TLM

HARVARD UNIVERSITY MONOGRAPHS IN MEDICINE AND PUBLIC HEALTH

Editorial Committee

Walter B. Cannon
A. Baird Hastings
James Howard Means
S. Burt Wolbach

LONDON : HUMPHREY MILFORD
OXFORD UNIVERSITY PRESS

THE ENDOCRINE FUNCTION OF IODINE

BY

WILLIAM THOMAS SALTER
Assistant Professor of Medicine, Harvard Medical School
Associate Physician, Thorndike Memorial Laboratory, Boston City Hospital

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE, * MYSORE. *
8 JAN 1951
LIBRARY
8 JAN 1951

CAMBRIDGE, MASSACHUSETTS
HARVARD UNIVERSITY PRESS
1940

RW 1133

COPYRIGHT, 1940
BY THE PRESIDENT AND FELLOWS OF HARVARD COLLEGE

L, 6 -
N40

CFTRI LIBRARY
1133

PRINTED AT THE HARVARD UNIVERSITY PRESS
CAMBRIDGE, MASS., U.S.A.

TO

CHARLES ROBERT HARINGTON

AND

JAMES HOWARD MEANS

JOINT MENTORS OF AN IATROCHEMIST

PREFACE

This volume was inspired by Professor A. Baird Hastings, who saw in the natural history of iodine an important bridge between biochemistry and endocrinology. The experience upon which it rests was gained in the laboratory of Professor Charles Robert Harington in London and in the clinic of Professor James Howard Means in Boston. For many decades iodine has attracted a great variety of professional interests — biological, physiological, chemical, medical, surgical. As the latter chapters will attest, the atomic physicist has recently joined the already cosmopolitan group. It is hoped that this community of effort is appropriately represented in bringing together here the many recent advances in studies of the halogen.

The general arrangement of the book is due to Mary E. Corcoran, the illustrations to Adelaide M. Hayes, and the bibliography to Eleanor Vallandigham Salter and Wilfrida Messenger. For many hours of their thoughtful work the author is greatly indebted. Acknowledgment is due also to the gracious assistance of the staff of the Harvard University Press and to the many authors, journals and publishers who have permitted unpublished data and copyrighted material to be reproduced. Finally, both the author and the reader owe much to Professor John P. Peters of Yale, who read large sections of the original manuscript and, with disarming candor, offered many helpful suggestions.

W. T. S.

Boston, Massachusetts
March 17, 1940

ACKNOWLEDGMENTS

The author wishes to thank those authors and publishers through whose courtesy the diagrammatic and tabular material reproduced in the text has been made available. The work of the following authors has been included:

H. Bauer and E. Strauss; E. J. Baumann and N. Metzger; F. Blum and E. Strauss; E. Brand, B. Kassell, and M. Heidelberger; J. W. Cavett, C. O. Rice, and J. F. McClendon; K. Closs, L. Loeb, and E. M. MacKay; E. J. Cohn; V. V. Cole and G. M. Curtis; G. M. Curtis and I. D. Puppel; L. Dautrebande; A. W. Elmer; G. L. Foster, W. W. Palmer, and J. P. Leland; M. Franke and L. Ptaszek; H. B. Friedgood; A. B. Gutman, E. M. Benedict, B. Baxter, and W. W. Palmer; K. Gutzeit and G. W. Parade; J. G. Hamilton and M. H. Soley; M. Heidelberger and K. O. Pedersen; S. Hertz and A. Kranes; S. Hertz, A. Roberts, J. H. Means, and R. D. Evans; G. E. Hudson; W. R. Ingram, C. Fisher, and S. W. Ranson; E. C. Kendall; G. Kuschinsky; M. A. Logan, J. E. VanderLaan, and W. P. VanderLaan, Jr.; J. F. McClendon; J. H. Means and J. Lerman; A. Neuberger; H. J. Perkin and B. R. Brown; H. J. Perkin and L. M. Hurxthal; H. J. Perkin, F. H. Lahey, and R. B. Cattell; E. P. Pick; R. E. Remington and H. Levine; D. S. Riggs and E. B. Man; G. Roussy and M. Mosinger; L. Scheffer; W. W. Smith and H. A. Ranges; P. Starr, R. W. Rawson, R. Smalley, E. G. Doty, and H. Patton; A. Sturm and R. Schneeberg; W. O. Thompson, A. C. Cohen, P. K. Thompson, E. G. Thorp, and A. G. Brailey; U. U. Uotila; H. B. van Dyke; G. B. Wallace and B. B. Brodie.

Grateful acknowledgment is made to the following publishers and scientific journals:

American Journal of Physiology, American Medical Association Press (including the *Archives of Internal Medicine*, *Archives of Pathology*, and *Journal of the American Medical Association*), *Archiv für experimentelle Pathologie und Pharmakologie*, *Biochemical Journal*, *Biochemische Zeitschrift*, *Endocrinology*, *Ergebnisse der Physiologie biologischen Chemie und experimentellen Pharmakologie*, *Journal of Biological Chemistry*, *Journal of Clinical Investi-*

gation, *Journal of General Physiology*, The Williams and Wilkins Co. (for the *Journal of Pharmacology and Experimental Therapeutics*), *Klinische Wochenschrift*, Masson et Cie (including *Comptes rendus des séances de la Société de Biologie*, *Congrès français de médecine*, and *La Presse médicale*), *New England Journal of Medicine*, Oxford University Press, Physiological Reviews, Reinhold Publishing Corporation (formerly Chemical Catalog Company), *Surgery, Gynecology and Obstetrics*, *Transactions of the Association of American Physicians*, University of Chicago Press, University of Minnesota Press, *Western Journal of Surgery, Obstetrics and Gynecology*, Wistar Institute of Anatomy and Biology (for the *Journal of Nutrition*), *Zeitschrift für die gesamte experimentelle Medizin*, *Zeitschrift für klinische Medizin*, *Zeitschrift für physiologische Chemie.*

Special acknowledgment is due also to the publishers of *Physiological Reviews* for permission to use, with minor modifications, large sections of the author's review, "Fluctuations in Body Iodine."

CONTENTS

I. IODINE BALANCE AND ENDOCRINE BALANCE . . . 3

II. IODINE STORES IN BODY TISSUES 6

TYPES OF IODINE COMPOUND IN THE ORGANISM 6
Iodide, 7; Naturally Occurring Organic Iodine Compounds, 7.

THYROID RESERVE 8
Fetal Thyroid, 13.

OTHER TISSUES 18
Pituitary, 19; Brain Tissue, 19; The Adrenal, 20; Gonads, 20; Viscera, 22; Synopsis, 22.

TEMPORARY IODINE STORAGE 22
Iodide, 22; Organic Iodine Compounds, 23; Thyroidal Trapping of Fresh Iodine, 24.

III. IODINE COMPOUNDS OF BIOLOGICAL IMPORTANCE . . 27

IODINATED PROTEINS 27
Hydrolysis, 29; "T" and "D" Iodine Fractions, 29.

NATURAL IODOPROTEINS 31
"Corals" and Sponges, 31.

THYROGLOBULIN 32
Preparation, 32; Properties, 34; Molecular Size, 35; Constituent Amino-Acids of Thyroglobulin, 37; Thyroglobulin, Normal and Pathological, 42.

ARTIFICIAL IODOPROTEINS 45
Preparation, 45; Physicochemical Peculiarities, 46.

THE IODINE-CONTAINING AMINO-ACIDS OF IODOPROTEINS . . . 50
Chemical and Physical Properties of Monoiodotyrosine, 51; Chemical and Physical Properties of Diiodotyrosine, 51; Chemical and Physical Properties of Thyroxine, 51; Other Possible Iodine-binding Amino-acids, 52.

SPLIT PRODUCTS OF IODOPROTEIN 57
Peptones and Plasteins of Thyroglobulin, 61.

ORGANIC IODINE COMPOUNDS IN RENAL CLEARANCE 64

IV. CIRCULATING IODINE 67

IODINE IN RESPIRED AIR 67

IODINE IN CEREBROSPINAL FLUID AND IN LYMPH 67
Exogenous Iodide in Cerebrospinal Fluid and in Lymph, 67.

IODINE IN MILK 70

IODINE IN SWEAT AND SALIVA 71

BLOOD IODINE 71
Physiological Range, 71; Qualitative Nature of Blood Iodine Compounds, 72; Extant Clinical Data, 76; Fasting Blood Iodine, 77; Effect of Exercise, 79.

Fluctuations in Blood Iodine, 81; Circulating Iodine after Administration of Iodine Compounds, 81; Iodide Administration, 81; Administration of Thyroxine Derivatives, 82; Thyroxine per Os, 82.

Hypothyroidism, 82; Assimilation of Iodine, 83; Effect of Thyrotropic Hormone, 84.

Hyperthyroidism, 84; Role of the Thyroid in Hyperthyroidism, 85; Relation of Blood Iodine to Basal Metabolic Rate, 86; Thyroidal Iodine, 89; Iodine Therapy, 89; Surgical and Radiation Therapy, 90; Thyroid "Storm," 91; Drugs and Hormones, 92.

Simple Goiter, 94.

Problems Concerning the Iodine of the Blood, 94.

V. THYROID ACTIVITY 100

Manufacture of Hormone 100

Topographical and Seasonal Variation, 101; Ebb and Flow of Thyroid Iodine, 103; Regulation of Iodine Turnover, 104; Endocrinological Regulation of Thyroid Activity, 107; Daily Production of Hormone, 107; Genesis of Thyroidal Activity, 109.

Calorigenic Activity of Thyroid Hormone in Various Forms 110

Thyroxine Derivatives, 111; Artificial Iodoproteins, 113; The Natural Hormone, 114; Potency of Thyroid Preparations, 115.

Thyroid Iodine Stores in Disturbances of Thyroid Function 118

Hypothyroidism, 118; Hyperthyroidism, 118; Iodine Therapy and the Hyperactive Thyroid, 119; Other Effects of Iodine, 122; "Jodbasedow" Effect, 122; Simple Goiter, 123.

VI. ENDOCRINE BALANCE 125

The Thyrotropic Hormone 125

Effect of Hypophysectomy, 126; Physiological Effects of Thyrotropic Hormone, 126; Concomitant Fluctuations in Iodine, 130.

The Pituitary-Thyroid Axis 131

(1) Opposing Effect of Circulating Hormone, 132; (2) Opposing Effect of Iodine, 133; Pituitary Iodine, 137; Pituitary-Thyroid Interaction, 139.

Thyroid and Other Endocrines 141

Adrenal Cortex and Iodine, 142; Thyroid and Parathyroid, 143; Pancreas, 143; Thymus and Thyroid, 143.

Peripheral Pituitary-Thyroid Relationships 144

VII. IODINE AND THE PITUITARY-OVARIAN AXIS . . . 146

Ovarian Iodine 148

Ovarian Cycle and Iodine Metabolism, 151; Ovarian Cycle and Iodine Excretion, 153; Pregnancy, 153.

Thyroid and Ovary 154

Reciprocal Thyro-Ovarian Relationships, 156; Effects on the Pituitary-Ovarian and Pituitary-Thyroid Axes, 156; Direct Thyro-Ovarian Interaction, 157.

VIII. NEUROLOGICAL INFLUENCE 160

Action of Thyroid Hormone on the Nervous System . . . 160

Action of the Nervous System upon the Thyroid . . . 162

Thyroid Nerves, 165.

DIRECT CONTROL OF THE THYROID 168

INDIRECT CONTROL OF THE THYROID 170
Neurohumoral Coordination, 171; Role of Iodine in Neurohumoral Integration, 175.

PERIPHERAL NERVOUS EFFECTS 179

IX. IODINE BALANCE 181

ASSIMILATION OF IODINE 182

Assimilation of Organic Compounds, 183; (1) Diiodotyrosine, 183; (2) Thyroxine and Its Derivatives, 184; Thyroxine in Peptide Combination, 184.

Iodine Requirements, 185; Minimum Normal Requirement, 186; Optimal Normal Requirement, 186.

Diet and Iodine Deficiency, 188; Conditioned Iodine Deficiency, 190.

Vitamins and Iodine, 190; Vitamin A, 190; Vitamin B Complex, 190; Vitamin C, 190; Vitamin D, 191.

Relative Iodine Deficiency, 191.

Prophylactic Dosage of Iodine, 192.

PHYSIOLOGICAL EXCRETION 192
Urinary Iodine, 192; Fecal Iodine, 193; Excretion by Other Routes, 194.

HUMAN IODINE BALANCE 194
Observations of von Fellenberg, 195; Observations of Scheffer, 196; Observations of Cole and Curtis, 197; Fasting Iodine Excretion, 200.

PHARMACOLOGICAL EFFECTS OF ADMINISTERED IODINE . . . 201
Iodide, 201; Renal Elimination, 202; Fecal Elimination, 202.

Organic Iodine Compounds, 202; Renal and Fecal Elimination, 202; Thyroxine, 203.

IODINE ELIMINATION BY THE LIVER 204
Organic Iodine Compounds in the Liver, 204.

THYROID DISTURBANCE 206
Hypothyroidism, 206; Hyperthyroidism, 208; Simple Goiter, 212.

X. RADIOACTIVE IODINE 213

RADIOACTIVE IODINES 215
"Labeled" Iodine, 216; Application of the Method, 217; Final Measurement of Radio-Iodine, 218; Preparation and Measurement of Short-lived Radio-Iodine, 218.

OBSERVATIONS WITH SHORT-LIVED RADIO-IODINE 219
Threshold for Accumulation of Iodine, 221; Observations with Multiple Labeling, 224.

EXPERIMENTAL RESULTS WITH I^{131} IN MAN 227
Gross Accuracy of the Method, 227.

RADIOACTIVE IODINE IN THE HUMAN THYROID 229
Distribution of Radio-Iodine in the Thyroid, 230; Radio-Iodine Measured in the Human Thyroid in Situ, 231; Critique of Radio-Iodine as a Physiological Method, 232; Radiation Therapy with Radioactive Iodine, 233; Element No. 85 (Eka-Iodine), 234.

XI. CLINICAL PROBLEMS 237

NATURAL LEVEL OF IODINE METABOLISM 239

Typical Problems, 240; Case 1. Normal, 241; Case 2. Simple Goiter with Possible Hypothyroidism, 241; Case 3. Anxiety Neurosis, 241; Case 4. Arteriosclerotic and Hypertensive Heart Disease, 242; Case 5. Auricular Fibrillation and Congestive Heart Failure, 243; Case 6. Toxic Adenomatous Goiter, 244; Case 7. Toxic Diffuse Goiter, 245; Case 8. Hypothyroidism in Pluriglandular Disturbance, 246; Case 9. Complete Myxedema, 247; Case 10. Thyroid "Storm," 247; Comment, 250.

PROPHYLACTIC LEVEL 253

THERAPEUTIC LEVEL 254

COMPLICATIONS OF IODINE THERAPY 256

Possible Exceptions to Routine Iodine Therapy in Hyperthyroidism, 259; (1) Cases of Graves' Disease with Normal Plasma "P" Iodine, 259; (2) Cases of Graves' Disease without Goiter, 260; (3) Medical Treatment per Necessitatem, 260; Poor Planning, 265; Iodine Tolerance, 266.

FIBROLYTIC LEVEL 268

APPENDIX FOR LABORATORY WORKERS 270

ESTIMATION OF IODINE 270

Amounts above 0.5 mg., 271; Hunter-Kendall Open Ashing Method, 271.

Amounts above 100 Micrograms, 273; Permanganate Method of Groák (I), 273.

Amounts above 10 Micrograms, 275; Micro-permanganate Oxidation of Groák (II), 275.

Amounts above 0.1 Microgram, 276; Wet-Ashing, 276; Permanganate Acid Ashing Method of D. S. Riggs and E. B. Man, 276; Electrometric Titration of McClendon, 283.

Separation of "T" and "D" Iodine, 284.

Determination of Thyroxine-like Iodine and Diiodotyrosine-like Iodine According to Leland and Foster, 285.

Analysis for Plasma Iodine: Directions for Separating "I," "P," "T," and "D" Fractions, 286.

ASSAY OF THYROTROPIC HORMONE 286

Method, 288; Results, 288.

SOME PROPERTIES OF IODINE 290

BIBLIOGRAPHY AND INDEX OF AUTHORS 293

SUBJECT INDEX 335

TABLES

1. Quantitative Determination of Iodine in Whole Gland and in Cells Free from Colloid Material of Human Thyroid Glands . 10
2. Iodine Content of Human Thyroids and Goiters 12
3. Amount of Iodine Absorbed by the Maternal and Fetal Thyroid in Rabbits 14
4. Iodine Partition of Thyroid Glands of Normal Individuals . . 15
5. Iodine Content of Normal Human Thyroids 16
6. Iodine in Various Tissues 21
7. Molecular Weight of Thyroglobulin from Sedimentation Equilibrium 36
8. Thyroxine and Diiodotyrosine Content of Thyroid Preparations 39
9. Molecular Weight of Thyroglobulin from Amino-acids and Combining Weights 40
10. Thyroglobulin and Degree of Follicular Hypertrophy . . . 43
11. Average Values Given by Thyroglobulin from Normal and Pathological Glands 44
12. Comparison of the pK Values of Tyrosine and Iodotyrosine . 47
13. Acid-Binding and Base-Binding Capacities of Zein and Iodozein 48
14. Constituent Amino-Acids of Protein which Bind Iodine . . . 54
15. Iodine Numbers of Some Artificial Iodoproteins 54
16. The Theoretical Stepwise Addition of Iodine to Globin . . . 56
17. Comparative Properties of Artificial Thyroid Protein and Nattural Thyroglobulin 63
18. Organic Iodine Compounds in Renal Clearance 65
19. Distribution of Iodine and Thiocyanate in Tissue and Blood . 68
20. Calculation of Body Fluid of Various Organs in which Iodide and Chloride are Distributed 69
21. Iodine Fractions in Blood 74
22. Range of Plasma Iodine in Boston 78
23. The "Iodine Quotient" in Normal and Hypertensive Individuals 87
24. Basal Metabolism and "Iodine Quotient" 88
25. Iodine in Desiccated Thyroid Glands of English Sheep . . . 102
26. Response to Thyroxine and Desiccated Thyroid 109
27. Effect of Large and Small Doses of Sodium Iodide on the Thyrotropic Activity of an Alkaline Extract of the Anterior Lobe of the Hypophysis 128

28. "Organische" and "Anorganische" Iodine in Thyroid Glands . 130
29. Effect of Various Urines upon Thyroid Cell Height in Guinea Pigs 133
30. Effect of Iodide on Thyrotropic Potency of Rat Pituitary . . 136
31. Iodine Content of Dog Brain 138
32. Iodine in Human Pituitaries 138
33. Iodine Content of Tissues of Dogs Receiving Iodine Daily . . 151
34. The Behavior of Blood Iodine Following Ovariectomy in Young and Old Bitches 152
35. Iodine Content of Human Brain 178
36. Iodine Excretion in Hyperthyroidism 187
37. Iodine Content of Soil and Goiter Rates in Switzerland . . . 189
38. Iodine Balance in Normal Man 199
39. The Iodine Balance in Diseases of the Thyroid Gland. A Comparison of the Iodine Balance of Normal Individuals with that of Nodular and Exophthalmic Goiter Patients . . . 209
40. Iodine Balance in Nodular Goiter, Nontoxic and Toxic . . 211
41. Isotopes of Iodine 215
42. Radioactive Iodine Studies in Normal Subjects and in Patients with Spontaneous Myxedema, and Various Types of Thyroid Disease 226
43. Plasma Iodine Findings in Ten Cases 248
44. Iodine Recoveries in Representative Micro-Iodine Determinations 283
45. Response of Acinar Cell Height to Increasing Dosage of Thyrotropic Hormone 289

FIGURES

1. Iodine Fractions in Human Thyroid 17
2. Trapping of Iodine by the Depleted Thyroid 25
3. Comparative Titration Curves of Zein and Iodozein . . . 49
4. Peptones, Peptides and Amino-Acids Contributing to Iodoprotein 58
5. Relief of Human Athyreosis by Iodoprotein 60
6. Proteolytic Cleavage Products of Natural and Artificial Thyroid Protein 61
7. "Organische" and "Anorganische" Blood Iodine Fractions Following Iodide Therapy 77
8. "Organische" and "Anorganische" Iodine Fractions in Human Blood 80
9. Blood Iodine and Basal Metabolism 86
10. "Organische" Blood Iodine after Thyroidectomy 92
11. Influence of Dietary Iodine upon the Thyroid 105
12. Requirement of Thyroid Hormone with Varying Metabolism . 108
13. Comparative Effectiveness of Thyroxine and Desiccated Thyroid in Normal Guinea-Pigs 115
14. Comparative Effectiveness of Thyroxine and Desiccated Thyroid in Human Myxedema 117
15. Effect of Extract of Anterior Pituitary on the Rabbit's Thyroid 127
16. Thyrotropic Hormone and Height of Thyroid Follicular Cells . 134
17. Antagonism between Iodide and Thyrotropic Hormone . . . 135
18. Urinary Excretion of Thyrotropic Hormone in Human Myxedema 141
19. Blood Iodine in Normal Dogs after Ingestion of Iodide . . 148
20. Blood Iodine in Pregnancy and after Thyroidectomy . . . 149
21. Blood Iodine after Oophorectomy 150
22. Innervation of the Pituitary Body 163
23. Probable Neural Interrelationships of the Hypothalamus and Pituitary of the Monkey 164
24. Influence of Hypophysectomy on Thyroid Activity . . . 169
25. Influence of Sympathectomy on Thyroid Activity 170
26. Iodine Balance in Normal and Thyrotoxic Individuals . . . 198
27. Iodine Balance in Human Myxedema 207
28. Trapping of New Iodide by the Rabbit's Thyroid 222
29. Functional State of the Thyroid and Fixation of New Iodide . 223

30. Fixation of New Iodide in the Thyroid Following Previous Iodide Administration 225
31. Urinary Excretion of Labeled Iodine 228
32. Direct *in Vivo* Measurements of Labeled Iodine in the Thyroid . 231
33. Response to Iodine Therapy in Human Exophthalmic Goiter . 257
34. Blood Iodine Fractions in Hyperthyroidism Following Iodine Therapy and Thyroidectomy 258
35. Blood Iodine Tolerance Curves in Man 259
36. Blood Iodine Fractions Following X-Ray Therapy . . . 262
37. Response of Pulse Rate to a Therapeutic Test of Iodine . . 264
38. Blood Iodine Tolerance Curves in Clinical Investigation . . 267
39. Distillation Apparatus for Analysis of Iodine 277
40. Histometric Assay of Thyrotropic Hormone 287

THE ENDOCRINE FUNCTION OF IODINE

CHAPTER I

IODINE BALANCE AND ENDOCRINE BALANCE

THE most important concept in endocrinology which emerges from the feverish activity of the past decade is the principle of endocrine balance. Discovered and rediscovered by several investigators, the paradoxical truth has dawned finally that man or beast may suffer less from the loss of several glands than from losing a single one. For each of the precious juices the other secretions supply a partial antidote, so that health and personality may be preserved, delicately poised. Henceforth the practicing consultant and the laboratory investigator, both, must think in terms of integrated hormonal effects and must look to precise chemical mechanisms whereby the parent organs react upon one another. No longer will it suffice to describe in romantic or in clinical terms the superficial results of imbalance. Even painstaking studies of general bodily metabolism and of hormone excretion will fail to touch the fundamental problem, because such studies record only ultimate effects. Interplay of endocrines must be described in terms of the glands themselves, especially in chemical terms. Thus, recent advances in knowledge of the sterols have served to emphasize the community in composition of the gonads and of the adrenal. These researches have tended to segregate reproduction and sex by assigning the former to organs which are dispensable and the latter to the adrenal cortex, which is indispensable to life. Nevertheless, in strictly chemical terms, there is good evidence that these hormones overlap and are interchangeable to some degree.

At best, however, this arrangement is only a minor entente in the endocrine system. More cogent is the balance of power throughout the entire league of endocrines. How is the hypophysial hegemony regulated and through what common mediator? No simple answer can be given to this question and certainly no complete description, but part of the problem can be projected in terms of iodine. Such an approach is justified by several findings. For example, the concentration of this element is phenomenally high in several glands, one of which is especially equipped to trap and to store it. In the athyreotic organism, other glands lose their iodine as they cease to function and regain iodine as health is restored. Moreover, at least one hypophysial tropic activity is profoundly influenced by iodine. For these and other reasons, iodine balance commonly reflects endocrine balance.

Since the discovery of iodine by Courtois in 1811,[107] two events stand out in our unfolding knowledge of iodine in relation to physiological processes. These are the discovery of iodine in the thyroid by Baumann in 1895,[39] and the isolation of crystalline thyroxine by Kendall on Christmas Day, 1914,[271] but in themselves these discoveries contributed little to the understanding of biological mechanisms. They are supremely important because they served as jumping-off points for painstaking researches by scores of other students who, bit by bit, have brought our knowledge to its present pitch. The chief impediment to these studies has been a lack of suitable techniques and, in particular, of analytical methods. This continues to be true, largely because of the very low concentrations of iodine found in most biological systems. The point is evident from a comparative study of successive classical monographs bearing on the subject, such as those of Kendall, 1921,[273] von Fellenberg, 1926,[168] Scharrer, 1928,[476] Harington, 1933,[220] Elmer, 1938,[139] Leipert, 1938,[312] and McClendon, 1939.[377] To these sources we owe much of our present knowledge about the usefulness of iodine balances and also about their limitations.

In 1850 Rabourdin [447] published his method for determining iodine, and forthwith there began a series of investigations which can be traced through Chatin [83] and von Fellenberg [167] to the present day. Although Bubnow, 1884,[68] identified the thyroid colloid as protein in nature, it remained for Baumann, 1896,[40] and Hutchison, 1896,[259] to study thyroglobulin as an *iodo*protein. Subsequently the identification of 3,5 diiodo-l-tyrosine by Wheeler and Jamieson in 1905 [574] established a working hypothesis for the protein's constitution which led to the isolation of the amino-acid by Harington and Randall [226] (1929). Meanwhile, Harington and Barger, 1927,[223] established that thyroxine also is a derivative of tyrosine. Finally, in the last decade it has become clear that the thyroid hormone is stored in a characteristic colloid form, containing at least two iodo-amino-acids in peptide linkage (Harington and Salter, 1930).[229]

In recent years attention has been focused upon the measurement of the minute amounts of iodine in the blood, in excreta, and in various tissues other than the thyroid. Amounts as low as 0.5 microgram (0.000,000,5 gram) have been measured within an error of 0.1 microgram, an accuracy satisfactory for many physiological problems.* Such procedures, however, demand a considerable analytical skill. Indeed, only a few years ago, several microchemists analyzed samples of normal blood and reported values for iodine ranging from 8 to 69 gamma per cent.[466] Fortunately, more reliable techniques are now available. Even though they are still crude, considering their objective, nevertheless they have been developed already to the point of dividing the vanishing biological iodine into characteristic fractions, i.e., "organic" and "inorganic."

Inasmuch as we allow the modern methodologists no place in our opening chapter, and indeed, banish them to the appendix, it must be emphasized that the study of iodine balance is peculiarly dependent upon rigorous technique. In fact, most of the information to be presented must be evaluated primarily on the basis of the analytical procedures involved. Because we are so patently at the mercy of the analytical chemists, as we close we shall pause to greet them, just inside the back cover.

---

* A microgram is 1/1000 milligram. In this volume the term is often used when the absolute amount or total content of a tissue or organ is stated. On the contrary, whenever concentration is mentioned, that is, micrograms per 100 grams of tissue, the term gamma per cent (or γ %) has been employed, in deference to common laboratory usage. Therefore, the purist will read micrograms per cent in place of gamma per cent.

CHAPTER II

IODINE STORES IN BODY TISSUES

JUSTUS, 1902–1904,[267] decided that all cells contain iodine, and in recent years reliable quantitative evidence has been adduced in favor of his point of view. Nevertheless, the iodine content of the whole human adult is small; it varies between 20 and 50 mg.[529] This amount is equivalent to less than 10 minims of liquor iodi compositus, U. S. P. Of this whole quantity, the muscles contain one-half, the skin one-tenth, and the skeleton one-seventeenth.

The total iodine in the blood of normal, fasting individuals is probably less than 10 gamma per cent, and contributes less than one-tenth of the whole iodine in the body. The human thyroid normally holds at least one-fifth of the total iodine, even though its mass is only one five-hundredth that of the whole body. Thus the thyroid iodine (40,000 gamma per cent) is a thousand times as concentrated as muscle iodine (30 gamma per cent). Lower values for all tissues may be encountered when the iodine supply is very low, but the normal thyroid maintains its capacity for the preferential trapping of iodine even when the amount of ingested iodine is greatly reduced.

The next highest iodine concentrations are found in other glands of internal secretion.[85] These include the anterior pituitary,[138] epiphysis, ovary, adrenal cortex,[157] and the parathyroid. In general, the iodine concentration of these endocrine organs is some fourfold that of other body tissues, which contain only about 20 to 30 gamma per cent. The increased concentration in the endocrines largely disappears after thyroidectomy,[529] whereas the iodine in skeletal muscle and other tissues remains relatively undisturbed. This fact raises the possibility that iodine in these endocrine glands is of thyroidal origin, a question to be studied further.

TYPES OF IODINE COMPOUND IN THE ORGANISM

At the present writing, tissue iodine usually is classified as (*1*) inorganic iodide, and (*2*) organically bound iodine, which may be (*a*) like thyroxine, insoluble in dilute aqueous acid, or (*b*) like diiodotyrosine, relatively soluble in dilute aqueous acid.

Iodide. It is clear that most body fluids contain inorganic iodide, the concentration of which (roughly, 2 gamma per cent) will be discussed later. Likewise, red cells are known to contain inorganic iodide. These concentrations, it will be noted, are rather low under ordinary physiological conditions. They may possibly be increased several times in hyperthyroidism, and certainly after the therapeutic administration of iodide over an hundredfold (see Chapter IV).

Pincussen and Roman [440] studied the electrodialyzable iodine in the mixed tissues of white mice. The gross average for iodine was 3.4 mg. per cent, of which the ratio of organic to presumably ionized iodine was 1.85. Such figures for iodide represent merely the total mass of tissue, and neglect the possibility that some of the diffusible iodine might not be iodide; they represent, therefore, maximal possible values. In the thyroid itself, relatively little inorganic iodide is found, even after recent administration of iodide to the organism,[220] when it may reach 10 per cent of the total thyroidal iodine, only to disappear rapidly after the medication is assimilated.

Naturally Occurring Organic Iodine Compounds. The other chemical combinations of iodine are iodinated tyrosine and iodinated thyronine. When elementary iodine is fed, as in liquor iodi compositus, U. S. P., it is soon changed in the gastrointestinal tract either into inorganic iodide or possibly, by combination with protein split products, into diiodotyrosine. Likewise, the iodine-poor human thyroid fixes circulating inorganic iodide in the form of diiodotyrosine soon after its administration.[210] Recently Hertz, Roberts, Means, and Evans [241] have studied the fixation of radioactive iodine and have found that the process may be nearly complete within fifteen minutes after a single dose, given intravenously. Subsequently, iodine is found in the form of tetraiodothyronine (i.e., thyroxine). Whether the intermediary stage, diiodothyronine, is present in the thyroid gland is still unknown.

Studies of artificially iodinated proteins [421, 37] suggest that other iodine compounds should be looked for in mammalian tissues. Indeed, monoiodotyrosine has been described [344] in hydrolysates of iodinated casein. Likewise, Bauer and Strauss [36] found evidence of iodohistidine (both the mono- and diiodo compounds). From the standpoint of chemical evolution, it should be noted that diiodotyrosine ("iodogorgonic acid") occurs in sponges and in "corals." [125] The origin of thyroxine in the evolutionary scale still remains to be studied carefully,* but much is known about its chemical genesis, as indicated in the next chapter.

THYROID RESERVE

By microdissection methods, fresh thyroid follicles may be "teased" apart and from them the intrafollicular colloid may be withdrawn as a viscid, colorless, albuminous liquid.[459] The crude colloid in the follicle is adulterated with varying amounts of nucleoprotein.[81] This intrafollicular nucleoprotein, however, has no known physiological function and is thought to originate from disintegrating or secreting cells of the follicular wall.

In addition, Williamson, Pearse, and Cunnington [576] have found evidence that two types of colloid can be isolated from the same gland. They used the technique of Tatum and of van Dyke, as described below. In this way they were able to separate colloid from different parts of the same piece of tissue, and to test this separated colloid by analysis of its iodine content and by bio-assay of its activity in producing metamorphosis of tadpoles. Thus they showed that portions of the gland which are commonly termed "inactive" apparently produce a secretion which is different from that found in follicles ordinarily termed "hyperplastic." Surprisingly enough, the "inactive" secretion, so-called, contained an abundance of iodine and, when added to water in which tadpoles were living, produced a rapid shrinking of the animals' tails and development of the limb buds. On the contrary, the colloid obtained from "secreting" tissue, so-called, showed very little iodine and essentially no activity by the tadpole test. From a pathological standpoint this finding is rather alarming, because it throws considerable doubt upon the ability of the routine pathologist to estimate pre-existing function of the gland under scrutiny by careful microscopic examination. From a chemical standpoint the results could be interpreted in at least two ways: first, hyperplastic tissue might conceivably "secrete" uniodinated protein which would subsequently be converted into active hormone during its stay within the follicle; or, secondly, perhaps follicles which are rapidly producing hormone for the general circulation abstract iodine from their stored colloid, thus leaving deiodinated protein behind. At present there is no good way of distinguishing between these two possibilities, but the problem urgently requires further investigation because of its physiological and pathological importance.

---

* "The now known endocrine glands, with the single exception of the gonads, which far antedate all other members of the series, begin to appear in the third class, the leptocardii; and in the eighth class, the amphibia, the series is complete. This corresponds roughly to the early primary era from the beginning of the Ordovician up through the Silurian and Devonian to the early Carboniferous epoch." [209]

Years ago, Tatum,[536] and later van Dyke [557] introduced a technique for separating the stored colloid from the parenchyma of the gland. In brief, this consisted in making sections of the frozen gland which were thin enough so that the central plugs of thyroglobulin could be dissolved out or floated out. The framework of parenchymal tissue, including both the stroma and the lining of follicular cells, could then be removed grossly and the gland could thus be separated into (*a*) a cellular mass and (*b*) a solution of thyroid colloid. By this method van Dyke [557] was able to demonstrate that the parenchyma contained a definite amount of iodine, although the bulk of the iodine was present in the form of colloid. The ratio of colloid iodine to parenchymal iodine was roughly six to one.

When this technique was applied to human goiters there was a greater variation in the distribution of iodine, as shown in Table 1. Nevertheless, the variability in ratio was much smaller than the variability in total iodine content of these glands. In short, goitrous enlargement seems not to involve any unusual upset in the distribution of iodine between cells and follicular lacunae.

If one supposes, however, that the connective tissue stroma, including nerves, blood vessels, and lymphatics, is relatively low in iodine, it seems not impossible that the thin layer of follicular thyroid cells, i.e., the true parenchyma of the gland, is very rich in iodine. Indeed, Uhlenhuth,[554] Sevringhaus,[505] and others have demonstrated droplets of colloid-like material forming within the cells prior to secretion into the follicle, much as mucin can be seen accumulating in the goblet cells of the respiratory tract. Furthermore, Williams [575] has described recently the removal of colloid from the follicle as a cellular phenomenon. He observed isolated follicles over a period of many hours, making serial observations on each of these small units. He found that not all of the follicles were active at one time, just as only certain of the renal glomeruli operate at the same time. While operative, however, the follicular membrane seemed to invaginate a small droplet of colloid at the edge of the follicle, then to wall it off by a protoplasmic bridge, and eventually

TABLE 1

Quantitative Determination of Iodine in Whole Gland and in Cells Free from Colloid Material of Human Thyroid Glands

Series no.	Weight of whole gland used	Iodine in whole gland	Weight of cell mass used	Iodine in cell mass	Ratio of percentage of iodine in cells to percentage of iodine in whole gland	Morphology		Diagnosis
						Cells	Colloid	
	mg.	%	mg.	%				
22	284.7	0.081	260.8	0.049	0.605	Cuboidal	Poor	Colloid cystic goiter with toxic symptoms
25*	600.2	0.274	308.6	0.105	0.383	Flat	Rich	Mild exophthalmic goiter
	411.0	0.276	240.7	0.098	0.355	......	....	
35	671.8	0.286	179.2	0.051	0.178	Flat	Rich	Toxic thyroid
36	439.7	0.158	219.9	0.020	0.127	Cuboidal	Fair	Toxic goiter
37†	667.5	0.092	258.4	0.027	0.293	......	....	Colloid goiter
38	578.6	0.079	191.7	Trace	....	Cuboidal	Poor	Colloid goiter following parenchymatous hyperplasia of exophthalmic goiter
39†	516.0	0.279	297.6	0.095	0.341	......	....	
40	469.2	0.345	225.5	0.066	0.191	Flat	Rich	Exophthalmic goiter
41	559.2	0.280	187.0	0.067	0.239	Flat	Rich	......
48*	549.9	0.043	312.4	0.005	0.116	Cuboidal	Poor	......
	607.3	0.044	311.3	0.005	0.114	......	....	......
49*	590.7	0.137	256.0	0.026	0.189	Cuboidal	Fair	Exophthalmic goiter
	...	....	204.5	0.027	0.197	......	.	......
51	731.4	0.283	294.4	0.072	0.254	Flat	Rich	Exophthalmic goiter
60	370.0	0.152	228.0	0.015	0.099	Flat	Rich	Cystic goiter with toxic symptoms

* Duplicate determinations made.
† No histological examination.

From: H. B. van Dyke, J. Biol. Chem. *45*: 330. 1920–1.

to release it into a capillary on the outside of the follicular wall by rupture of the thinned-out protoplasmic wall behind the droplet. If this mechanism be the true one, then the follicular lining will be high in iodine, both because of hormone which is being manufactured within the cells and because of colloid which is being passed backwards from the stored central reserve into the circulation. In other words, the six to one ratio observed by Tatum and by van Dyke [557] may represent simply the relative mass of the follicular thyroid cells to the stroma.

It will be noted in the next chapter that this general type of separation has been utilized in the preparation of the very pure samples of thyroglobulin studied by physical chemists in recent times. It affords an excellent opportunity to make observations upon the stored hormone, apart from the manufacturing cells. It would be of great interest, however, to develop this technique further by micromethods of both mechanical and chemical character so that the follicular cells might be studied from the standpoint of their iodine content and the type of iodine compounds which they contain at various stages of functional activity. Very recently Logan, VanderLaan and VanderLaan [341] have studied the distribution of water and electrolyte between cells and colloid. These authors have demonstrated a marked change from the normal status under the influence of thyrotropic hormone. Their findings are described briefly in Chapter VI.

Numerous observations upon the iodine content of various types of thyroid have been made since the time of Baumann.[39] In Table 2 are reproduced a few representative examples of his classical figures, both for human thyroids and goiters in clinical cases.

The iodine in the normal thyroglobulin, as well as in the whole tissue, may vary considerably, depending largely upon the supply of iodine available to the animal donor. In thyroglobulin prepared from the glands of Argentine sheep by the author, for example, the partially purified, heat-coagulated protein contained over 1.2 per cent iodine, of which over 60 per cent was apparent thyroxine. By contrast, average human thyroglobulin in Boston contained 0.22 per cent iodine, of which only 25 per cent was thyroxine-like.

In regions where goiter develops because the iodine supply of the food and water is low, there may be almost no iodine in a very large gland. When such an individual or animal is given an excess of iodide, however, the iodine content in the gland may increase very rapidly, as shown in Baumann's [40] old data and confirmed by many investigators since.[360, 577] Even when iodine supply is more than adequate throughout the general community, a person suffering from exophthalmic goiter may lose most of the iodine in the gland because of a relative iodine lack. When such an individual is treated with the relatively huge doses now in vogue in modern clinics, the iodine content of the gland may increase over twentyfold within two weeks.

TABLE 2

Iodine Content of Human Thyroids and Goiters

No.	Age (years)	Cause of death	Dry weight of thyroid	Iodine Content in Milligrams Per gram dried substance	 In entire gland
1	24	Pulmonary tuberculosis	21	0.30	6.3
2	34	Puerperal sepsis	24	0.07	1.7
3	44	Miliary tuberculosis	24	0.12	2.7
4	49	Nephritis	26	0.7	1.8
5	61	Cancer of stomach and liver	58	. . .	. . .
6	62	Fatty heart	24	Trace	. . .
7	66	Senile marasmus	23	Trace	. . .
8	67	Osteomalacia	44	0.1	4.4
9	70	Emphysema	23	0.3	6.9
10	63	Emphysema	80	0.05	4.0
			Weight in grams Fresh Dry		
11		Freshly extirpated colloid goiter	220 40	0.07	2.8
12		Freshly extirpated colloid goiter	165 42	0.02	0.84
		Average	. . . . .	0.09	2.6

From: E. Baumann, Ztschr. f. physiol. Chem. *22*: 9. 1896–7.

In the next chapter it will be shown that usually the thyroxine fraction within the gland disappears in greater proportion than does the total iodine. Indeed, in a huge colloid goiter furnished the author by Dr. F. A. Coller of Michigan, the total iodine in the thyroid protein was only 0.006 per cent and the thyroxine fraction thereof was essentially nil. Such material is of little value as a hormone, as proved by tests in animals or patients.[469] Although it has been suggested that ordinarily thyroxine is only lightly bound to this colloid vehicle,[201] it seems clear that l-thyroxine is liberated from firm union in the peptide chain by the action of proteolytic enzymes.[229] In normal thyroglobulin, both the diiodotyrosine and the thyroxine are in the l-configuration.[228, 229] It remains to be seen whether the protein of thyroid cancer contains the d- forms, as suggested by the work of Kögl and Erxleben.[291] The latest evidence seems *not* to favor this possibility.

Fetal Thyroid. Even the thyroid of the fetus reflects the general availability of iodine in the environment. Thus thyroids in fetuses of cretinoid mothers which abort are nearly iodine-free. Likewise, the glands of hairless pigs produced experimentally [571] by depriving brood sows of iodine (or by withholding auxiliary iodine feeding in an endemic goiter district) are poor in iodine when analyzed. Early in fetal life, the thyroid fails to show well developed follicular spaces and consists merely of massed alveolar groups or cords of follicular cells distinguished by only a small central lumen at best. When the mother is well supplied with iodine, however, these fetal thyroids contain an abundance of iodine. This chemical evidence is of interest in connection with the distribution between iodine and cells already mentioned in the work of Tatum and of van Dyke.[557]

Maurer [371] analyzed the thyroids of human fetuses from the seventh to the ninth month. His findings ranged from 37 to 1400 gamma per cent of fresh tissue. This material apparently represented in part active hormone which might supplement the maternal supply. Indeed, it has long been known that pregnant bitches may be thyroidectomized and continue healthy until after delivery. It is clear, too, from the work of Hudson [257] that the maternal supply of ingested iodide may pass through the placental circulation to the fetal thyroid. The data in Table 3 indicate also that under such external treatment with iodide the iodine concentration in the fetal gland may increase to more than twice its natural value (which averages 13 gamma per cent), although it soon reaches a limiting concentration. Marine and Lenhart [360] studied the thyroids of puppies at birth in a region naturally poor in iodine. Unless the mothers were supplied with extra iodide during gestation the glands of the offspring contained almost no iodine. On the other hand, when artificial sources of iodine augmented the maternal intake in the latter weeks of pregnancy, the young had considerable amounts of thyroid iodine. Under similar circumstances iodine may be detected also in the urine of the newborn child, as well as in the amniotic fluid.

Iodine appears in fetal pig and sheep thyroid between the second and fourth month of gestation. Likewise, after the fourth month the fetal human thyroid constantly contains iodine. The total iodine present in human tissue from the third to the ninth month varies from 1 to 19 micrograms. The concentration in dried tissue is 2 to 21 mg. per cent. In fresh tissue this probably amounts to from 0.18 to 4.6 mg. per cent, although Lelkes found lower values, i.e., from 0.5 to 152 gamma per gram. At least part of this iodine is already present in active form. Hogben and Crew (1923) obtained a positive Gudernatsch test, and Hektoen and Schulhof (1925) a positive precipitin test for thyroglobulin. Likewise, Elmer and Scheps,[156] by chemical analysis, found a thyroxine-like fraction in about the same proportion of the total iodine as in the protein of the adult. Indeed, thyroidectomized bitches have remained healthy while pregnant but later developed severe "cachexia strumipriva," the beginning of which could be detected a few days after delivery. The marked hyperplasia of the fetal thyroid under such circumstances indicates also that the mother is receiving fetal hormone through the placental circulation.

TABLE 3

Amount of Iodine Absorbed by the Maternal and Fetal Thyroid in Rabbits

Daily amount of KI in mg. given to experimental mother	I in γ Per 100 Mg. of Thyroid			
	Mothers		Fetuses	
	Control	Experimental	Control	Experimental
20	7	34	12	22
20	12	26	14	24
100	14	75	13	22
100	8	101	14	26
100	12	142	15	27
100	13	142	14	27
100	9	136	10	29
200	13	165	9	29
Mean	11.0	103	12.6	25.7

After: G. E. Hudson, J.A.M.A. *97*: 1515. 1931.

Newborn babies contain from 2.4 to 48 gamma of thyroidal iodine in percentage varying from 2.3 to 1450 gamma per cent of fresh tissue, whereas the other tissues contain only from 12 to 46 gamma per cent. In hogs Fenger (1912) found, in per cent (fat free), 0.11 to 0.32, and Smith (1917) 0.064 to 0.236 (dry); in sheep Fenger found 0.09 to 0.36. The ratio of thyroxine-like to total iodine is the same as in the adult. The thyroidal iodine increases in total amount from birth to puberty, at which stage it increases rapidly. It reaches a maximum at about the twentieth year. After the fiftieth year it begins to decline. Characteristic percentages for human tissue are given by Glimm and Isenbruch.[199] In the first year 24 mg. per cent, in the first decade 25, in the second decade 42, in middle life 29, after the sixtieth year 36 mg. per cent. In old age a hyperfunctioning thyroid may compensate for a decline in other functions.

TABLE 4

IODINE PARTITION OF THYROID GLANDS OF NORMAL INDIVIDUALS

Gland no.	Dry weight	Iodine Per Gram Dry Gland			Thyroxine I per cent of total organic I	Histology
		Total	Thyroxine	Inorganic		
	gm.	*mg.*	*mg.*	*mg.*		
1	6.21	0.57	0.11	0.06	21.6	Early involution
2	3.51	0.84	0.20	0.10	27.0	Normal, connective tissue increased
3	3.74	1.07	0.22	0.07	22.0	Normal
4	4.24	1.16	0.26	0.05	23.4	Normal, early involution
5	3.74	1.32	0.17	0.07	13.6	Early hyperplasia
6	9.13	1.56	0.30	0.08	20.3	Normal, connective tissue increased
7	4.27	1.65	0.26	0.08	16.6	Early involution, basophilic colloid
8	2.70	1.70	0.24	0.07	14.7	Early involution
9	7.93	1.71	0.43	0.10	26.7	Normal resting gland
10	3.02	1.73	0.40	0.32	28.4	Normal resting gland
11	3.08	1.82	0.57	0.11	33.2	Early involution
12	8.22	1.85	0.50	0.07	28.1	Normal resting gland
13	8.88	2.28	0.49	0.13	22.8	Normal
14	5.18	2.28	0.62	0.10	28.4	Normal
15	6.78	2.55	0.65	0.25	28.3	Normal resting gland
16	3.97	2.72	0.90	0.10	34.3	Normal resting gland
17	3.34	2.82	0.97	0.09	35.5	Normal resting gland
18	4.98	2.83	0.67	0.32	26.7	Normal
19	4.05	3.86	1.21	0.18	32.9	Normal resting gland
20	4.08	4.31	1.38	0.20	33.6	Normal resting gland

From: A. B. Gutman, E. M. Benedict, B. Baxter, and W. W. Palmer, J. Biol. Chem. *97*: 319. 1932.

The adult thyroid contains 20 per cent of all the iodine in the body, even though its average weight is only about 20 grams. Nevertheless, over half the total of the iodine in the body is found in the great mass of skeletal muscle, which contains about 13 mg.[529] The iodine content of the thyroid tends to increase after birth, reaching a maximum at about forty years of age. As shown by Gutman, Benedict, Baxter, and Palmer,[210] the average total weight of normal thyroids in New York was about 25 grams of fresh tissue, or 5 grams of dry substance, containing 8.85 mg. of iodine. Even in these so-called normal organs, there was a considerable range of concentration, i.e., from 0.05 to 0.45 per cent of dried gland, averaging 0.186 per cent, as shown in Table 4 and Figure 1. These analyses were made with thyroids from individuals not treated with iodine.

The actual mass of the thyroid in middle age varies in different localities. In Zürich Herzfeld and Klinger found 50 grams and Oswald (1902) 95 grams. In Lwów Elmer and Salzer (1935) found 30 grams on the average; in Munich Jansen and Robert (1927) reported about 30 grams; in Oslo Lunde, Closs, and Wülfert [347] found 23 grams. Of course the weight varies with the degree of hyperplasia and retention of colloid, even in apparently normal individuals.

TABLE 5

Iodine Content of Normal Human Thyroids

Author	Country	Number of glands examined	Iodine in terms of dried gland %
Zunz	Belgium	56	0.229
Pellegrini	Italy	111	0.100
Jolin	Sweden	108	0.156
Wells	U. S.	108	0.210
Marine	U. S.	23	0.217
Average			0.182

From: L. Dautrebande, Physiopathologie de la thyroïde. Congrès français de médecine. *21*: 62. 1930.

Although the iodine content of various localities throughout the world varies widely, as shown by McClendon,[377] the average content of dried glands from various parts of the world is not very different from that required by the United States Pharmacopeia for thyroidea sicca, i.e., close to 0.2 per cent. This fact is illustrated in Table 5 by the comparative data from Dautrebande.[120]

It is not known definitely in what chemical form the hormone is released from the colloid stored in the follicle. By enzymic hydrol-

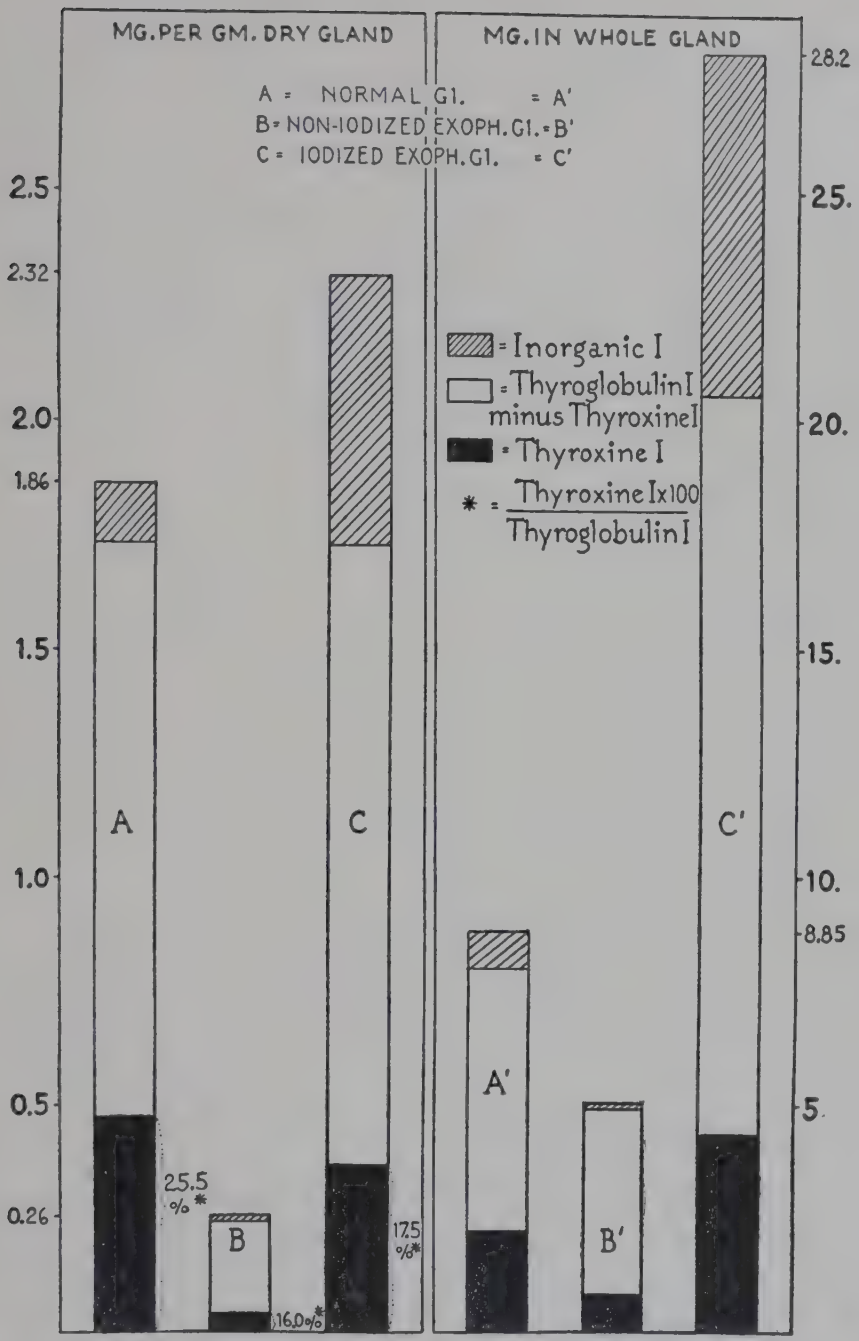


FIG. 1. The average total iodine, "T" iodine, and inorganic iodine in human thyroids from (*a*) euthyroid individuals, (*b*) hyperthyroid patients not treated with iodine, and (*c*) hyperthyroid patients treated with iodine. Data marked A is derived from work of Leland and Foster combined with that of Gutman, Benedict, Baxter, and Palmer; data marked B from Wilson and Kendall.

From: A. B. Gutman, E. M. Benedict, B. Baxter, and W. W. Palmer, J. Biol. Chem. 97: 320. 1932.

ysis Harington and Salter [229] obtained a peptide preparation of thyroxine which was assayed in human myxedema by Salter, Lerman, and Means.[472] Its greater solubility suggested that it might resemble the true circulating hormone. Further assays suggested that the remaining non-thyroxine iodine in thyroid protein also contributed to its potency in human myxedema. Harington [221] summarized this paradox by suggesting that the active thyroid secretion consists of a peptide chain containing both thyroxine and other amino-acids, including diiodotyrosine. This problem is interesting from a therapeutic standpoint and will be discussed further in Chapter V.

OTHER TISSUES

Not much is known about the state of the organically bound iodine in other tissues. There is a thyroxine-like fraction, "T," in the blood,[149] and probably also a diiodotyrosine-like fraction, "D." [549] Neither of these fractions is supported by a positive chemical identification, although the "T" fraction has been confirmed by bioassay.[583]

Direct attempts to isolate thyroxine from tissues at the present time are complicated not merely by difficulties in technical manipulation, but also by a more fundamental and, at present, surprising difficulty. This complication may be summarized as follows:

In recent years it has been found that such a nondescript protein as casein, when iodinated and subjected to alkaline hydrolysis, yields compounds related to thyroxine.[5, 344, 471] Indeed, even unhydrolyzed serum albumin, after iodination, relieves human myxedema in classical fashion.[321] The method of isolation involved in treating these nondescript proteins is quite similar to that for isolating thyroxine from thyroid protein. Consequently, the recovery of thyroxine from any tissue might be merely the result of an artefact. An alternative interpretation would be that organically bound iodine in the body is always in thyroxine-like form, a supposition which is by no means suggested by the classical studies of artificial iodoproteins.[523, 36]

Biological considerations, however, indicate obviously that some thyroxine derivative circulates from the thyroid to the tissues by way of the blood stream. Moreover, hormonal activity has been demonstrated in tissue. Thus Kommerell [292] fed muscle meat obtained from both normal and myxedematous animals. The latter flesh failed to increase oxygen consumption in test animals, although the normal flesh did so. Likewise, tissue extracts have been studied from the standpoint of effect on metamorphosis.[2] Thus Zawadowsky and Perlmutter[585] have used the metamorphosis of axolotl to amblystoma to detect the presence of thyroid hormone. This biological phenomenon occurs completely with as small a dose of thyroxine as 10 gamma per 10 grams of body weight. Accordingly, when iodine concentration is rigidly controlled, the confusing effects of other iodine compounds can often be excluded. When this test is performed carefully, therefore, it is more specific than the Gudernatsch[208] tadpole method or Uhlenhuth's procedure,[554] and offers greater hope for detecting thyroidal activity in tissue extracts or in body fluids. By this method, for example, Zawadowsky and Asimoff[584] were able to demonstrate increased hormonal activity of the liver, kidney and blood serum in guinea pigs which had received thyroid preparation about six hours previously.

Unfortunately, such attempts to estimate concentrations of hormone are at present in the pioneer stage. Improved micromethods for detecting thyroid hormone in blood and other tissues are badly needed.

Pituitary. The total iodine concentration of the fresh mammalian anterior pituitary is about 80 to 190 gamma per cent,[87] and of fresh human (whole) pituitaries about 80 gamma per cent iodine, or 490 gamma per cent of dried substance.[529] In commercial desiccated anterior pituitary powder, between 50 and 87 per cent of the total iodine is in organic combination.[87] The pituitary protein, or "colloid," contains about 1.3 mg. per cent iodine.[411] This protein merits further study because it is said that the "colloid" is secreted into the venous circulation of the pituitary. Furthermore, the high concentration per se, if verified, must have a peculiar significance. Baumann and Metzger[41] recently have obtained lower results which they explain as due to improved analytical technique. This question is discussed further in Chapter VIII.

The posterior lobe of the hypophysis is low in iodine, containing only 40 gamma per cent in desiccated tissue.[295] This fact suggests that it does not participate directly in iodine metabolism. Indeed, after thyroidectomy no histological changes appear in the posterior lobe, although such changes are characteristic in the anterior and middle lobes.[139, p. 293]

Brain Tissue. Despite contradictory reports,[343] the iodine concentration in the diencephalon * probably attains much higher values than in other portions of the cerebrum, i.e., between 347 and 708 gamma per cent.[495, 439] More work is needed to decide whether the diencephalon specifically concentrates this iodine from the blood or gets it indirectly from the pituitary. Likewise, it remains to be decided whether the tuber cinereum and surrounding regions really do absorb thyroid hormone selectively. Schittenhelm and Eisler,[496] as well as Pighini,[439] say yes. Sturm and Schneeberg [530] and Löhr and Wilmanns say no. Meantime, it must be confessed that certain positive evidence is highly suggestive. For example, within a few weeks after hypophysectomy, the naturally high concentration of the tuber cinereum drops to 14 gamma per cent.[528] Conversely, after large doses of "thyroidin" † the iodine concentration in the pituitary may rise to 2400 gamma per cent and that in the tuber cinereum to 564 gamma per cent.[343] Furthermore, injections of anterior pituitary may increase the interbrain iodine from 666 to 2690 gamma per cent.[439] Further details are given in Chapter VIII.

The Adrenal. The dried adrenal cortex of beef contains from 26 to 66 gamma per cent of iodine, whereas the dried medulla has only 16 to 20 gamma per cent.[157] Since the mass of the cortex is greater than the medullary tissue, the cortex of one average ox adrenal contains from 0.7 to 0.8 microgram as against 0.2 to 0.3 microgram in the medulla. Thus the whole adrenal (weighing 10 or 12 grams) contains about 1 microgram of iodine. Surprisingly enough, there seems to be no appreciable "thyroxine-like" material in either part of the ox gland,[157] when analyzed by Leland and Foster's [315] method. Apparently not all members of the sympathetic system accumulate thyroxine-like stores! Smaller animals have proportionately more adrenal iodine. Representative values are, for the dog, 4 gamma [157] to 5 gamma [529] per single gland; for the guinea pig, 0.1 gamma for both glands [167] at a concentration of 18 gamma per cent in fresh tissue.

Gonads. The ovaries rank third among the tissues of the body with respect to iodine concentration. In women the iodine content ranges from 30 to 160 gamma per cent of fresh tissue; it may be as high as 741 gamma per cent.[529, 375] In the fresh ovaries of animals it ranges from 60 to 2150 gamma per cent for rabbits,[374] as high as 340 gamma per cent for guinea pigs,[529] and from 104 to 161 gamma per cent for dogs. In human newborn it is lower, averaging 138 gamma per cent; it is highest during the child-bearing period and declines after the menopause. It probably varies during the ovarian cycle.[246, 78] Indeed, the corpora lutea are especially rich in iodine.[418] Next to the thyroid, the ovaries of dogs receiving iodine trap the most iodine per gram of tissue.[429] More precise information is given in Chapter VII.

---

* The casual reader of the general literature is likely to be confused by the rather widespread use of the term "midbrain" when the diencephalon is meant, i.e., the hypothalamus.

† "Thyroidin" is essentially a peptone derived from thyroglobulin and contains iodine organically bound both in the form of thyroxine and of diiodotyrosine. The term usually refers to desiccated thyroid or thyroid extract.

TABLE 6

Iodine in Various Tissues

	Sturm[526] Man		Sturm and Buchholz[529] Dog		Maurer and Ducrue[374] Rabbit
Organ	γ %	Abs.	γ %	Abs.	γ %
Thyroid	29400–29256	10234–5266	8500–19500	170–580	1170–33000
Liver	102–118	1340–2030	39–170	107–1300	2–18
Skeletal muscles	30	3000	39–61	1390	..
Skeleton	30	3000	17–22	305	..
Skin	150	5250	12–148	179	4–17
Ovaries	30–160	..	104–161	..	60–2150
Testicles	165	33	25–54	4	..
Pituitary	80	..	..	..	..
Epiphysis	120	..	..	..	..
Parathyroids	385	..	..	..	..
Adrenals	296–393	33–38	187–530	5	..
Pancreas	44	29	41–65	6–8	..
Thymus	..	..	46–57	3	..
Spleen	335–429	302–420	53–136	14–82	26–160
Submaxillary gland	550	88	..	..	12–94
Kidneys	20	40	18–37	5–9	4–51
Lungs	24	144	22–33	23–26	4–10
Uterus and fallopian tubes	..	..	..	..	70–160
Stomach	..	..	..	..	..
Small intestine	35	490	12–31	52	20
Colon	..	..	..	..	4
Heart	77–100	188–330	32–75	22–85	4–49
Hypoderm	23	3450	..	91	9
Brain	12	163	12–27	9–30	1.5
Hair	133	400	..	..	8–53
Fingernails	..	..	..	..	27

After: A. W. Elmer, Iodine Metabolism and Thyroid Function. London. 1938. Oxford Univ. Press, pp. 82–83.

The chemical nature of the ovarian iodine is unknown. Carter[78] found in the ripe eggs of echinus esculentus a substance which produced a marked effect on tadpoles. This problem needs further study.

Unlike the values for ovarian iodine, the absolute values for total testicular iodine are small: i.e., for man 3.3 micrograms; for dog 4 micrograms; and for cattle 2 micrograms.[167] The male gonad, unlike ovary, is low in iodine, e.g., from 7 to 10 gamma per cent iodine in fresh dog testicles,[139, p. 329] and in human glands 17 gamma per cent.[529]

Viscera. Most of the viscera show as low an iodine content as skeletal muscle unless material containing iodine in quantity has been administered recently. Thus the fresh whole pancreas contains only 13 to 20 gamma per 100 grams of fresh tissue.[138] * In dogs, therefore, the corresponding total amount of pancreatic iodine is only 6 to 8 gamma.[529] A possible exception is the liver, which participates specifically in the metabolism of iodine compounds, as discussed in Chapter IX. The liver of man has been found to contain from 520 to 2300 gamma,[139, p. 190] and that of the fasting guinea pig from 107 to 150 gamma. Less than one-tenth of this consists in "thyroxine-like" iodine, i.e., 200 gamma per cent in desiccated liver substance,[9] but the actual nature of the liver iodine is not known definitely.

Synopsis. Although there is still considerable disparity between the analyses given by various investigators for the iodine in tissues, it is of some interest to survey relative concentrations published by several authors, noting that these undoubtedly will be subject to considerable revision within the next decade. Such comparable values are shown in Table 6.

TEMPORARY IODINE STORAGE

When iodine is administered from an external source, there results a temporary state of metabolic flux which alters the characteristic concentrations just described. These alterations vary with the source of extra iodine.

Iodide. The fate of inorganic iodide after absorption depends in part on the dosage used and on the physiological state of the recipient. Maurer and Ducrue[374] gave rabbits 10 gamma iodine per kilogram in this form and analyzed various tissues both one day and four days later. They found a great increase in the thyroid iodine, corroborating the classical findings of Marine [352] in the iodine-hungry dog. The liver, skin, and lungs showed very high iodine contents as temporary storage depots, but after four days only the heart, kidneys, and lungs remained unusually rich in iodine.* Increased concentrations have been found temporarily also in the stomach and parotid.[326] Obviously, the organism does not retain extra iodide long. With larger doses in guinea pigs, the thyroid stores only 4 per cent of the extra iodine accumulated, but the skin, hair, muscles, and bones store iodine for many days at a high concentration.[168] The lungs and trachea show very high concentrations temporarily, but soon lose the extra iodine despite the fact that the expired air shows no increase in iodine content.[374]

---

* To what extent the islet tissue participates in the total is unknown.

Organic Iodine Compounds. Simple organic iodine compounds likewise accumulate in the skin.[334] Furthermore, after three weeks' daily administration, iodotryptoflavin is stored especially in the brain and kidneys.[167] Experiments with diiodotyrosine are unsatisfactory because as yet there is no micromethod available for determination of traces of this compound.

The fate of thyroxine derivatives, however, has been studied both by iodine analysis and by the bio-assay method of Zawadowsky and Asimoff.[584] Asimoff, Estrin, and Miletzkaja [26] studied the effect of a single dose of "thyroidin" by mouth in dogs and roosters. They found that iodine accumulated in the thyroid, kidneys, liver, blood, pancreas, skin, heart, and spleen. Increased iodine is found likewise in the muscles and bones of the rat five days after a single intravenous injection of thyroxine.[297] Small amounts of this iodine remain stored for a long time in the animals' tissues, especially in the liver, muscles, thyroid, and skin.[400] In another section the selective accumulation of thyroxine iodine by the hypophysis has been discussed.[530] Whether the tuber cinereum shows this in even more marked degree [495] has not yet been decided.

The biological method of Zawadowsky has yielded interesting information as to the fate of thyroxine derivatives in bodily economy. Zawadowsky and Asimoff [584] fed "thyroidin" to mammals and birds and estimated its concentration in various tissues by bio-assay. During the first few hours the hormone is trapped in the liver to a large extent. Thereafter it increases in the blood plasma and in the kidneys. After twenty-four hours the hormone largely disappears. Birds appear to destroy or assimilate the hormone less rapidly than mammals.

---

* The distribution of this iodide throughout body fluids has been studied by Wallace and Brodie,[568] as discussed in Chapter IV.

Thyroidal Trapping of Fresh Iodine. It is not clear that inorganic iodide is present in the thyroid of a normal animal receiving no iodine. At most it might be 10 per cent of the total present.[227] Elmer [138] found that it might amount to from 0.36 to 0.63 mg. in the whole thyroid, or from 2.7 to 9.8 mg. per cent in dried tissue. However, the present technical methods yield rather different answers for inorganic iodide, depending upon the treatment which the gland receives. In extracts of fresh sheep glands the author found well over 90 per cent of the soluble iodine to be heat-coagulable.

Immediately after the administration of therapeutic doses of iodine to the intact organism the thyroid (unless previously satiated) traps a considerable portion of the iodine within a few hours. At this stage, inorganic iodide may account for over 30 per cent of the trapped iodine. This inorganic moiety, however, is largely converted into organic form in a day or two.[361] This process has been studied recently by Hertz and Roberts [241] who used radioactive iodine, as described in Chapter X.

Of the total iodine, then, normally nearly the whole is organically bound. Of this, Meyer [395] found 2 per cent as lipoid. The rest is in protein form, combined in the thyroglobulin. From the thyroglobulin, two iodo- amino-acids have been isolated, diiodotyrosine, and thyroxine, as described elsewhere.

The relative proportion of thyroxine-like substance in the thyroid varies more than the total iodine. This is true because this fraction is prone to disappear faster both when excessive amounts of hormone are withdrawn from the gland and when there is a relative iodine deficiency. All of the methods in vogue involve alkaline hydrolysis which destroys part of the thyroxine. On the other hand, such hydrolysis may leave a little diiodotyrosine still linked to thyroxine. Consequently the analytical results are only approximate, but they are remarkably consistent.

Using fresh human thyroglobulin preparations, Salter and Lerman found that 20 per cent of the total iodine was thyroxine-like by Leland and Foster's method or by Blau's method, whereas 25 per cent was the figure obtained by Harington and Randall's [227] procedure. Although the latter method involves compensating errors, it is simpler and (the author believes) more truthful for many purposes. As shown in Figure 2, Gutman, Benedict, Baxter, and Palmer [210] found much the same proportion for human goiters, surgically excised. Their data would suggest that under treatment with iodine the normal proportion between "T" and "D" iodine fractions quickly is restored in the glands of patients suffering from exophthalmic goiter. Brand and Kassell [61] report similar values by their recently developed colorimetric method. By Leland and Foster's method, Elmer (1936) found from 3 to 5 mg. of apparent thyroxine in the whole thyroid, Leland and Foster (1932) 0.2 to 9 mg. and Gutman (1932) 0.9 to 7 mg. In dog thyroids Elmer found 0.7 to 1.1 mg. apparent thyroxine, and in rabbits only 3 to 5 micrograms.

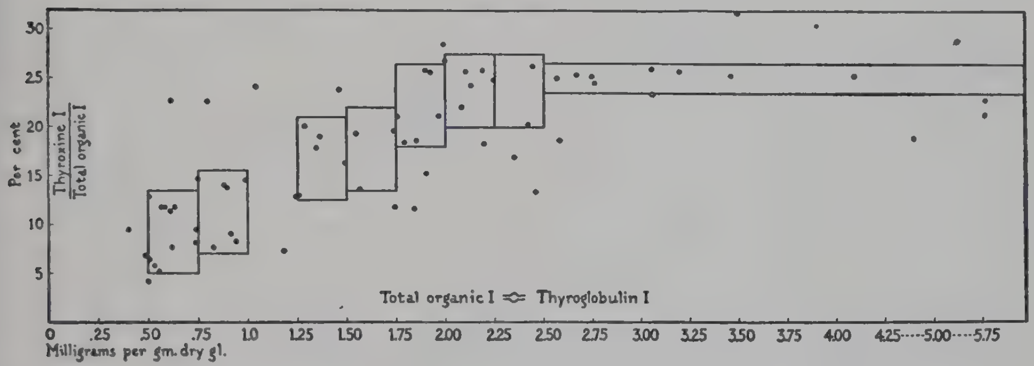


FIG. 2. As the exophthalmic goiter responds to iodine therapy, the total iodine in the gland increases. In the first few days the relative proportion of thyroxine-like "T" iodine rises steadily; later it assumes a constant proportion of the total iodine. This trend is indicated by lines drawn to include areas containing the majority of points, which represent analyses of seventy iodized glands from human cases of exophthalmic goiter.

From: A. B. Gutman, E. M. Benedict, B. Baxter, and W. W. Palmer: J. Biol. Chem. *97*: 315. 1932.

Grab [201] separated the colloid in the follicles from the cellular constituents and analyzed both for apparent thyroxine. He found that in both parts the relative proportion of apparent thyroxine was the same, i.e., about one-third.

Diiodotyrosine accounts for about two-thirds of the iodine in the thyroid. This fraction tends to persist when the thyroxine-like fraction is depleted. Elmer found (in this form) from 2.0 to 6.1 mg. of iodine in human thyroids, 0.2 to 1.19 mg. iodine in dog thyroids and from 4 to 18 micrograms in rabbit thyroids. This figure usually includes the inorganic, which is often presumed to be negligible. As stated in Chapter III, this substance is presumably the precursor of thyroxine.

There seems to be no question that the colloid consists largely of thyroglobulin, which may be regarded as the storage form of the hormone as well as the specific thyroid protein. Unlike insulin in the pancreas, however, which apparently has a definite sulfur content, thyroglobulin varies in its iodine content and in the relative proportions of diiodotyrosine and apparent thyroxine. This variation will be discussed in detail in Chapter III.

CHAPTER III

IODINE COMPOUNDS OF BIOLOGICAL IMPORTANCE

Although organic chemists know thousands of pure compounds containing iodine, the materials which are of interest to biologists and to physicians are largely derivatives of protein; in fact thyroglobulin, the colloid of the gland, is the best known iodoprotein. The ancient Greeks had some inkling of this fact in their treatment of goiter with "burnt sponge," but no sort of scientific approach to this problem was clear until therapeutic use by Coindet [94] of the pure element about 1811. The striking chemical activity of iodine naturally led to a systematic investigation of its chemical properties, and in 1876 Böhm and Berg [53] noted that iodine could combine with egg white and that subsequently this iodine could be removed readily. In the next year Knop [286] treated egg albumin with bromine and obtained a brominated derivative thereof. Following the discovery by Baumann [39] in 1895 that the thyroid protein was an iodoprotein, many investigators were attracted to the study of natural combinations of iodine with protoplasm or constituents of it. This widespread interest led to the discovery of iodoproteins in sponges by Hundeshagen (1895),[258] and in "corals" by Drechsel (1896).[125] A few years later Baumann conducted an intensive attempt to increase the activity of thyroid protein by further iodination of the natural material, and Hutchison [260] hydrolyzed natural thyroglobulin with proteolytic enzymes to obtain iodinated peptones.

Iodinated Proteins

This pioneer work opened up the general field of organic albuminous derivatives which has been pursued along two general lines, namely, the artificial iodination of well-known proteins to form homologous iodoproteins, the properties of which can be studied; and also the isolation in pure form of the split products of both natural and artificial iodoproteins. Among the pioneers who laid down the foundations of our present knowledge were Hofmeister, Oswald, and Blum. Indeed, from Blum through Strauss and Bauer to the present day one traces three generations of work in this field. Although Drechsel hydrolyzed the horny axial skeleton of the Mediterranean "coral," Gorgonia cavallini, by means of alkali, and obtained from it a crystalline split product which he called iodogorgonic acid, it was not until 1911 that Oswald was able to isolate this substance from several proteins and to identify it as diiodotyrosine. Nearly twenty years later Harington and Randall finally succeeded in obtaining the same material from thyroglobulin [226] and eventually established our concept of "D" iodine — a term which will be used frequently in this volume.

In the meantime, methods for introducing iodine artificially into protein molecules had been developed further. In the early days Hofmeister [248] used a strongly acid medium for iodinating the dissolved protein, but his method was not very satisfactory because the proteins were partially split in the process. Blum and his collaborators later tried various methods of iodination and eventually came to use bicarbonate to buffer the iodinated medium. They recognized two kinds of iodine in the resulting iodoproteins, the one tightly bound to carbon, the other loosely bound to nitrogen. Blum and Strauss [48] considered that the iodine bound to nitrogen joined the NH group of the imidazole ring of histidine, and they found that this iodine could be split off by sulfurous acid. They concluded that in general the carbon-bound iodine consisted mainly of diiodotyrosine. They found, however, that certain proteins, notably globin, bound more iodine than could be taken up by the tyrosine residues therein. The clue to this paradox was furnished by Pauly,[427] who found in 1910 that iodine could combine with histidine derivatives by entering the imidazole group. Thereupon Bauer and Strauss [36, 38] succeeded in showing that the total iodine in iodoglobin could be accounted for by assuming that the histidine group contained the iodine not accounted for by tyrosine. Thus the story of iodo-histidine has run parallel to the history of iodotyrosine, and it remains to be completed fully by the isolation and synthesis of pure iodo-histidine.

In recent years the properties of iodoproteins have been compared with those of the homologous proteins before iodination. Both Cohn [92] and Neuberger [407] have found that the dissociating hydroxyl of tyrosine becomes more strongly acid on the addition of two atoms of iodine. Similarly, Haurowitz [231] found that, after iodination, globin can no longer combine with hemin to form hemoglobin.

Furthermore, Cohn, Salter, and Ferry [93] found by comparing globin with iodoglobin that the base-binding power of the latter is lower, a fact which suggests that the hydroxyl groups in the tyrosine residues have become blocked in some fashion. Because these artificially iodinated proteins bear many resemblances to natural iodoproteins, and to thyroglobulin in particular, their properties are of peculiar interest.

Hydrolysis. The hydrolysis of both natural and artificial iodoproteins has been a very fruitful line of investigation. Following the pioneer peptic and tryptic digests of Hutchison [259] in 1896, various attempts have been made to break up these proteins into their constituent iodo-amino-acids.[247] This work not only led to the isolation of diiodotyrosine, as already mentioned, but it eventually produced thyroxine in 1915. This substance Kendall obtained from the thyroid by alkaline hydrolysis, after years of acid hydrolysis had failed. In recent years this same procedure, namely, the use of barium hydroxide as a hydrolytic agent, has led to startling results with artificial iodoproteins. The preliminary experiments were performed by Abelin,[5, 6, 9] who found that artificially iodinated casein, when hydrolyzed, produced effects which mimicked those of the thyroid hormone. Later, Ludwig and Mutzenbecher [344] isolated pure crystalline thyroxine from such digests. Furthermore, Salter and Lerman have shown that simple iodination of the proteins of mammalian blood serum, e.g., serum albumin, yields iodoprotein which relieves in classical fashion patients suffering from typical clinical myxedema.

"T" and "D" Iodine Fractions. These investigations, therefore, have served to describe the origin of thyroidal activity in terms of classical chemistry, and have shown that in many respects the natural protein of the thyroid resembles material produced in the laboratory. It can now be inferred that natural endocrine activity must originate in consequence of chemical reactions which may be duplicated *in vitro*. In other words, this series of researches has successfully bridged the gap between endocrinology and the older chemistry of nutrition. Moreover, these studies have led to the recognition of two general fractions of iodine combination in natural materials, namely, the "D" fraction already mentioned as resembling and containing diiodotyrosine, and also a second, "T" fraction, which resembles and contains thyroxine or derivatives thereof. Hutchison in the last century noted that peptic digestion of thyro-

globulin yielded such fractions, and ten years ago Harington and Salter pursued this division further by the use of pepsin, trypsin, and erepsin successively to isolate levorotatory thyroxine and also the levo− (+) diiodotyrosine (Harington and Randall, 1931). In recent years Salter and Lerman[469] have tested such subfractions of natural human thyroglobulin and have shown that the hormonal activity of the protein remains qualitatively with the "T" fraction as the molecule is subdivided progressively into smaller and smaller pieces. That is to say, after each fission of the molecule the "T" moiety has been found to relieve human myxedema, whereas the "D" fraction has been shown to be inert. Likewise, iodinated blood proteins have been split into iodopeptones, iodopeptides and into iodo-amino-acids; again the thyroidal activity of these artificial substances has remained in the "T" fraction.

When one comes to the iodine of the circulating blood, — present in a concentration so small as to tax present analytical methods to their utmost, — it is still possible to show that the organically bound iodine is precipitated along with the plasma proteins. Contrary to earlier evidence, under ideal conditions relatively little of the native iodine is salted out with the globulin fractions in the preliminary separation. It is not yet clear whether this circulating organic iodine is truly colloidal or whether it is merely adsorbed on the plasma colloids. Possibly the circulating iodine is a proteolytic split product derived from the huge thyroglobulin molecules deposited in the natural thyroid colloid. Indeed, Perkin and Hurxthal[431] have recently added to normal human blood such a fragment prepared by Salter, and found that this fortified blood behaved like naturally hyperthyroid blood, whereas normal blood fortified with crystalline thyroxine did not reproduce the natural behavior. Whether colloidal or not, however, it is clear that the circulating iodine in human blood consists in both a "T" and a "D" fraction, and recently Salter has used the same procedure in separating these fractions as that formerly employed with the homologous fractions of thyroglobulin and of artificial iodoproteins. It remains to be seen how these circulating "T" and "D" iodine moieties react in endocrine imbalance, especially in thyroid disturbance.

Already it is clear that the total organic iodine of the blood is high in hyperthyroid states. Under certain conditions this total organic iodine decreases when a patient is successfully treated by the flooding of his body fluids with tremendous excesses of inorganic iodide. In some clinical conditions, however, an abnormally high concentration of organically bound iodine exists in the absence of demonstrable hyperthyroidism. Does such organic iodine consist chiefly of "D" iodine? Does the "T" iodine really determine the degree of thyroid activity? Is a drop in "T" iodine the true chemical correlative of clinical improvement in hyperthyroidism? Is the "T" fraction absent in frank myxedema, even though the concentration of total iodine and of organically bound iodine be within normal limits? Such questions are the obvious extrapolations of problems which already have been answered, and the possibility of pursuing them will be considered in subsequent chapters. Before proceeding to these general problems, however, it will be necessary to examine in greater detail the technical aspects of the materials and methods involved.

NATURAL IODOPROTEINS

"Corals" and Sponges. In the last century a systematic search for natural iodine compounds was conducted and it was observed that most of these substances were related to the sea, if not to modern oceans at least to those of some prehistoric geological era. In addition to sponges and "corals" or sea fans, it was soon noted that kelp and seaweed were also rich in iodine. In all of these organisms the iodine could be divided by chemical means into inorganic iodide and into iodine in organic combination.[237] As early as 1848 Vogel showed that sponges contained organic iodine; forty-seven years later Hundeshagen studied this combination further and concluded that the iodine was present in the form of an amino-acid. Harnack [230] described in greater detail the protein of the sponge skeleton as an iodo-albuminoid and named it iodospongin. Subsequently Drechsel prepared an insoluble protein from corals and isolated from hydrolysates thereof his "iodogorgonic acid," later shown by Wheeler and Mendel (1909–10) to be diiodotyrosine. Although these researches led ultimately to the intensive study of thyroglobulin by Baumann, this last signal event was purely accidental.

It is amusing to read Baumann's original account of this incident, now more than forty years past, in which he says "als ich diese Beobachtung zuerst machte, glaubte ich an alles Andere eher, als dass Jod meiner Substanz angehöre." [39] He had known, of course, about the researches of Bubnow, who, about 1875, isolated crude thyroid protein by extracting minced thyroid glands with salt solutions. This work was later pursued by Hutchison,[259, 260] who extracted the glands first with five per cent magnesium sulfate solution and later (and better) with one-tenth per cent sodium hydroxide. When the solution was cautiously acidified with acetic acid, Hutchison obtained a flocculating protein which had the properties of a globulin, and which he recognized as the protein in the thyroid colloid. In his day the old theory that the thyroid manufactured mucin still held sway, but Hutchison hydrolyzed his product with hydrochloric acid and found no evidence of mucin. He also isolated a small amount of nucleoprotein from his extracts, but rightly decided that it was derived from the thyroid cells and that it was not, properly speaking, a true thyroid secretion. In retrospect, it is remarkable how much enduring and fundamental information this pioneer English investigator published in two physiological reports.

Thus only three naturally occurring iodoproteins are well known, namely, spongin from sponges; gorgonin from sea fans (erroneously called "corals"); and thyroglobulin from the thyroid of vertebrates. Although it is true that seaweed may contain 0.5 per cent of iodine, no pure iodinated proteins have been isolated from this source; Toryu found only 5 per cent of the iodine of seaweed combined with protein. Of course this small amount could be accounted for by simple "adsorption," i.e., formation of a salt with free groups of the protein.

THYROGLOBULIN

Preparation. Until recently the best characterization of the active thyroid protein has consisted in a series of papers by Oswald, the first of which was published in 1899. He found that practically all the iodine in the thyroid could be extracted with physiological salt solutions and that nearly all the iodine so extracted was chemically bound and, in fact, precipitable by heat coagulation. He treated such solutions with ammonium sulfate and found at half saturation with this salt that the globulins so precipitated had carried down nearly all of the iodine. Oswald purified this globulin fraction further by dissolving it in dilute alkali and reprecipitating it cautiously with dilute acetic acid. This method has been used repeatedly with minor modifications even to the present time. One of the best preparations of thyroglobulin yet made was that of Heidelberger and Palmer,[232] who used Oswald's method of repeated extractions with nine-tenths per cent aqueous sodium chloride. These authors were able to remove most of the contaminating nucleoprotein by adjusting the reaction of the very cold thyroglobulin solution to pH 4.8 or 5.0. After this purification they precipitated the thyroglobulin either with sodium chloride or with ammonium sulfate at half saturation. They attempted an acid extraction with dilute acetic acid, as formerly proposed by Oswald, but they found that this method denatured the thyroglobulin.

For many purposes, extraction of the glands with dilute alkali is convenient, but it tends to hasten the proteolysis, and for purposes of careful study by physical chemists this alkaline medium has been avoided. Accordingly, Cavett and Seljeskog (1933) have in recent years advocated the very simple procedure of extracting the sliced frozen gland with 1 per cent aqueous sodium chloride solution. This extract is filtered and adjusted to pH 7.4; it is then fractionally precipitated with ammonium sulfate. The fraction precipitating at 35 to 45 per cent saturation is saved. For purposes of analysis the material may be freed from lipids by precipitating the thyroglobulin at $-5°$ C. with acetone, and subsequently extracting the (denatured) protein in a Soxhlet's apparatus with ether for forty hours.

Thus far, these two carefully purified preparations of mammalian thyroglobulin are probably the best which have been produced. In neither case was it possible to free the thyroglobulin completely from phosphorus, although in the later preparations the phosphorus content dropped to less than 0.02 per cent. Apparently there is no proof that thyroglobulin contains phosphorus, but as yet no one has obtained a preparation completely free from it. Subsequently Cavett, working with Rice and McClendon,[81] applied his method both to hog thyroid and to human thyroids obtained at autopsy or at operation. He pointed out that the impurities in thyroglobulin preparations are mainly ammonium sulfate, nucleoprotein and fat. Because the nucleoprotein usually is derived by destruction of the cells of the gland, grinding of the tissue was avoided; the tissue was merely frozen and sliced. Later the fat precipitates with the thyroglobulin, but it is completely removed if thyroglobulin is precipitated with acetone and extracted in a Soxhlet's apparatus with pure ether. (The precaution must be taken of placing some metallic sodium in the flask; otherwise the thyroglobulin will be denatured.) For example, when hog's thyroglobulin was precipitated with ammonium sulfate, it contained 0.51 per cent iodine, but after being dissolved in water, precipitated with acetone, and extracted for twenty hours in a Soxhlet's apparatus with anhydrous ether, it contained 0.65 per cent iodine. On further redissolving in water, precipitating with acetone, and extracting with ether, it contained 0.697 per cent iodine. This great increase in the percentage of iodine was due to the removal of fat.

The same general procedure can be applied to human thyroid. When nucleoprotein is present initially in low concentrations, — and this can be attained by using sliced thyroid rather than ground tissue, — the nucleoprotein impurity can be removed by repeated dissolving of the thyroglobulin in water and reprecipitation with ammonium sulfate between the limits of 35 and 45 per cent saturation.

Out of thirty-three preparations so compared by Cavett, Rice, and McClendon, nineteen had a phosphorus content between 0.01 and 0.03 per cent. It seems probable, therefore, that about 0.02 per cent of the phosphorus is contained in the thyroglobulin and that larger amounts of phosphorus are indicative of an admixture of nucleoprotein. Because this iodoprotein, like the muscle protein, is rather labile, strongly acidic reactions must be avoided in order to prevent denaturation. In fact, Heidelberger and Palmer[232] found that in attempting to remove nucleoprotein they almost invariably denatured their thyroglobulin by going to reactions more acid than pH 4.5.

Properties. The general properties of this native protein, first outlined by Hutchison and later described in detail by Oswald, have been confirmed by the more careful studies of recent years. The protein is only slightly soluble in water, but it is more soluble in dilute salt, and still more so in dilute alkali. Indeed, it possesses all of the characteristics of a globulin, and forms a globul*an* on the addition of acid to saline solutions, much as the vegetable globulins are precipitated by this means. It has been known for a long time that this protein is coagulated by heat, and when all the nitrogen is removed thus from its pure solutions, all the iodine is coagulated simultaneously. It is known also that this material is very low in phosphorus. As far as can be determined, essentially all of the pharmaceutical potency inherent in the substance of the gland is extracted in this protein form. Unfortunately, at present no good studies of titration curves, that is, of acid-binding and of base-binding, are available for very pure thyroglobulin preparations. Likewise solubility studies have been performed only in a most cursory fashion. Such careful studies would be extremely interesting. At present, all one can say is that thyroglobulin has an isoelectric point close to 4.5 and that the pH value for the isoelectric zone shifts to a less acid value when the material is denatured. Inasmuch as denaturation is progressive, this shift also may be progressive until a complete denaturation is reached (with isoelectric values of about pH 5).

In general, the precipitation limits for thyroglobulin with ammonium sulfate indicate, of course, that the material behaves somewhat like pseudoglobulin of plasma, but this characterization is at best crude and it would be desirable to have more precise data. That the material, when undenatured, behaves like a globulin is indicated clearly from the fact that it dissolves readily in 1 per cent sodium chloride solution and that this solubility is increased if the solution is made more alkaline. In fact, starting with an alkaline chloride solution, the solubility of thyroglobulin progressively diminishes to pH 4.5 and then increases somewhat at extreme acidity if this change in pH be made so rapidly that a chance for denaturation and coagulation is avoided. The same sort of phenomenon has been described in the case of the vegetable proteins and for muscle globulin, as described by Salter.

Molecular Size. The original preparation of Heidelberger and Palmer was tested in the laboratory of Thé Svedberg for its molecular mass, using the ultracentrifuge at Upsala.[234] Although the original values so attained were over 700,000, this figure has been revised more recently by Heidelberger and Pedersen and stated to be about 675,000. The methods used, of course, were the well known methods of Svedberg[533] for determining the molecular weight of proteins. The procedure is too complicated to describe here, but it depends upon the determination (*1*) of diffusion constants and (*2*) of sedimentation constants as found in the ultracentrifuge. Indeed; the ultracentrifugal technique permits a direct determination of the molecular weight by means of sedimentation equilibrium measurements. The following formula has been used:

$$M = \frac{RT \frac{dc}{dx}}{(1 - V \cdot \rho)\omega^2 \cdot x \cdot c},$$

where M equals molecular weight; R is the thermodynamic gas constant; T is temperature (absolute); V equals specific volume; ρ equals density of the solution; ω equals angular velocity of the centrifuge; x equals distance from the center of rotation, and c equals concentration in grams per milliliter at the distance x, as determined by refractometric measurements. By this method Heidelberger and Pedersen found the values given in Table 7 which, when properly calculated, yield the molecular weight in question.

TABLE 7

MOLECULAR WEIGHT OF THYROGLOBULIN FROM SEDIMENTATION EQUILIBRIUM

c at Start = 0.970; pH = 6.6

Revolutions per second	Distance x	Concentration c	Molecular Weight M
49.5	5.55	0.421	686,000
	5.60	0.513	657,000
	5.65	0.623	638,000
	5.70	0.755	627,000
	5.75	0.915	630,000
	5.80	1.115	661,000
	5.85	1.390	(772,000)
Average			650,000
42.5	5.55	0.520	667,000
	5.60	0.599	656,000
	5.65	0.690	649,000
	5.70	0.799	653,000
	5.75	0.926	684,000
	5.80	1.092	(790,000)
Average			662,000

After: M. Heidelberger and K. O. Pedersen, J. General Physiol. *19*: 105. 1935–36.

Their results may be summarized as follows:

1. The sedimentation constant of hog thyroglobulin is 19.2×10^{-13} sq. cm. per second. That of human thyroglobulin is essentially the same.

2. The specific volume of hog thyroglobulin is 0.72.

3. The isoelectric point of native hog thyroglobulin is at pH 4.58, that of denatured thyroglobulin at pH 5.0.

4. The molecular weight of hog thyroglobulin is, in round numbers, 700,000, as calculated from the sedimentation and diffusion constants, or 650,000, as calculated from the sedimentation equilibrium data.

5. The thyroglobulin molecule deviates markedly from the spherical.

More recently Svedberg in Sweden and Williams in Wisconsin have noted that by adjusting the concentration of urea and by varying the pH in the solutions the thyroglobulin molecule seems to divide, first, into two pieces and, later, into four in a reversible fashion. These results [532] indicate that the thyroglobulin as determined originally was an aggregate; indeed, we have no definite notion as to what its real state is in the thyroid colloid as naturally deposited.

Constituent Amino-Acids of Thyroglobulin. A further estimate of molecular size has been made by using the principle of minimal combining weights, in association with common modern theories of protein structure. This was done with Heidelberger and Pedersen's [233] highly purified preparation of thyroglobulin from glands of Swedish hogs. Part of this same preparation, denatured by heat, was analyzed by Brand, Kassell, and Heidelberger [62] for its constituent amino-acids, and through the kindness of the authors the data are summarized here. Because the carbohydrate content of individual thyroglobulin preparations varies considerably, the relative percentages obtained in a single preparation are more reliable than analyses involving material obtained from distinctly separate sources. Even so, it will doubtless be possible to correct separate series of analyses so that they may be superimposed for comparison.

Brand, Kassell, and Heidelberger undertook not merely to determine the percentage content of the individual amino-acid components of the purified thyroid colloid, but they also studied their results from the standpoint of protein structure, in accordance with theories developed in the last few years by Svedberg [533] and by Bergmann.[44] Briefly stated, they undertook to decide between two alternative possibilities: namely (*a*) the thyroglobulin molecule is a haphazard jumble of amino-acids, bound together in peptide linkage; or (*b*) the molecule has an orderly, systematic arrangement and consists of definite units arranged in stoechiometric relationship to one another.

The analyses are a tribute to the photometric techniques developed by Kassell and Brand.[269, 270] After hydrolysis with alkali or with alkaline stannite, various amino-acids were studied quantitatively. It was observed that diiodotyrosine and thyroxine gave no Millon's reaction either before or after hydrolysis with alkali, but during hydrolysis with alkaline stannite both compounds yielded active phenols. Accordingly, diiodotyrosine and thyroxine were determined indirectly in the hydrolysates by estimating the extra phenol-like substance which the stannite treatment produced. On the assumption that

$$\text{Total iodine} = \text{“D” iodine plus “T” iodine}$$

the extra phenolic color could be allocated properly by using appropriate equations for the tyrosine equivalents of diiodotyrosine and thyroxine, respectively, i.e., 0.713 and 0.286 per milligram of iodine. Thus the

$$\text{“D” iodine} = \frac{\text{the extra phenol} - 0.286 \times \text{total iodine}}{0.427}$$

and, by difference, the "T" iodine could be estimated. When this method was used, the values for thyroxine and diiodotyrosine shown in Table 8 were obtained in this highly "pedigreed" (and therefore very significant) material.

It is to be hoped that similar data may be obtained for human thyroglobulin, both normal and pathological. At any rate, these values for the "D" iodine and the "T" iodine show clearly that the major part of the thyroglobulin iodine is not present in the form of thyroxine. This point will be discussed in detail in a subsequent chapter because around it have centered two major discussions involving the physiological and pharmaceutical importance of the "D" iodine. The first of these questions, briefly put, is this: Does diiodotyrosine represent the natural precursor of thyroxine? The second question, briefly stated, is: Has the "D" fraction pharmaceutical potency? These two problems have been the source of a considerable amount of research in the past few years, and the evidence is so complex that it must be deferred for discussion at greater length.

Brand, Kassell, and Heidelberger went further and compared several individual amino-acid residues in thyroglobulin, together with the glucosamine and carbohydrate radicals, as shown in Table 9.

TABLE 8

THYROXINE AND DIIODOTYROSINE CONTENT OF THYROID PREPARATIONS CALCULATED AS EXPLAINED IN THE TEXT

Prepa-ration no.	Method of analysis	Total iodine %	"Extra tyrosine" %	THYROXINE %	THYROXINE As iodine %	THYROXINE Per cent total iodine	DIIODOTYROSINE %	DIIODOTYROSINE As iodine %	DIIODOTYROSINE Per cent total iodine
1	Brand and Kassell	0.425	0.23	0.25	0.17	39	0.44	0.26	61
	Leland and Foster	....	...	0.19	0.12	29	...	...	..
	Harington	....	...	0.33	0.21	50	...	...	..
2	Brand and Kassell	0.36	0.19	0.24	0.16	44	0.34	0.20	56
	Leland and Foster	....	...	0.15	0.10	27	...	...	..
3	Brand and Kassell	0.142	0.09	0.03	0.02	16	0.20	0.12	84
	Leland and Foster	....	...	0.01	0.01	5.4	...	...	..

From: E. Brand and B. Kassell, J. Biol. Chem. *131*: 500. 1939.

TABLE 9

MOLECULAR WEIGHT OF THYROGLOBULIN FROM AMINO-ACIDS AND COMBINING WEIGHTS

$$N_i = \frac{M_p}{M_i} \times \frac{\%}{100} \qquad N_t = \frac{N_i}{F_i} \qquad \frac{M_p}{R} = \frac{665{,}000}{115.3} = 5768$$

Constituent	Per cent Found	Residues				Per cent calculated for different molecular weights			
		Number N_i		Fraction F_i	Total N_t				
		Found	Corrected			650,000	665,000	675,000	695,000
Cystine (½)	4.30	238	240	1/24	5760	4.43	4.33	4.27	4.14
Methionine	1.31	59	60	1/96	5760	1.38	1.34	1.32	1.29
Total protein sulfur	1.46	303	300			1.48	1.45	1.42	1.38
Tryptophane	1.88	61	60	1/96	5760	1.88	1.84	1.81	1.76
Tyrosine	3.00	110	110	1/52.3	5756	3.07	2.99	2.95	2.87
Diiodotyrosine	0.64	9.8	10	1/576	5760				
Tyr. + diiodo.	3.27	120	120	1/48	5760				
Thyroxine	0.32	2.7	2 or 3			Sum of deviations of the above found values from calculated			
Total iodine	0.58	30.4	(28 or 32)						
						0.29	0.12	0.20	0.51
Glucosamine	2.2	81.7	80	1/72	5760				
Carbohydrate	3.45	127	120	1/48	5760				

From: E. Brand, B. Kassell, and M. Heidelberger, J. Biol. Chem. *128*: xi. 1939 (Scientific Proc.).
(Data by courtesy of the authors.)

To these data they applied some of the well known conventions of analytical chemistry, as summarized by the following definitions and equations:

N_i = number of residues of an individual constituent
N_t = total number of residues
$\frac{N_i}{N_t} = F_i$ = fraction of total residues
M_p = molecular weight of protein
$\frac{M_p}{N_t} = R$ = average residue weight
$\%$ = per cent by weight of constituent in protein
M_i = molecular weight of constituent

Accordingly, $M_p = \frac{N_i}{F_i} \times R = \frac{100}{\%} \times M_i \times N_i$

and $\frac{1}{F_i} = \frac{100}{\%} \times M_i \times \frac{1}{R}$. Hence $R = 115 \pm 8$ (for most proteins).

Using these formulae it was possible to calculate the number of residues, N_i, from the percentage figures on the basis of a molecular weight of 665,000. These values then could be corrected to the nearest integer indicated by the theory of Bergmann.[44] Likewise, for each constituent it was possible to calculate its fraction of the total residues, F_i, as shown in the table. These various figures lead ultimately to the conclusion that there exist 5760 constituents in the thyroglobulin molecule. Consequently, these investigators suggest that each thyroglobulin molecule is composed of ten double-Svedberg units [533] of 576 constituents each, and with polysaccharide attached to each double-unit.

This interpretation of the structure of thyroglobulin no doubt rests upon a highly theoretical basis. Nevertheless, it is derived in accordance with the best modern theories of protein structure, and is illuminating because it indicates that the framework of thyroglobulin may be a true biochemical entity, albeit a complicated one.

This point of view was extended by estimating various values for the molecular weight, as predicted from the theoretical number of residues contributed by each constituent. It will be observed that, of four values tried, the value 665,000 best fits the data actually obtained (see Table 9).

Thyroglobin, Normal and Pathological. As far as the iodine content is concerned, as well as its partition into thyroxine-iodine and diiodotyrosine-iodine, Cavett, Rice, and McClendon[81] were able to study the thyroglobulin extracted in the fashion just mentioned from a series of different types of glands. They studied the iodine content of the protein as obtained at autopsy and from thyroidectomized individuals who had *not* suffered from obvious thyroid disease. In addition they compared three types of goiter, namely, the colloid goiter, the so-called adenomatous, or nodular goiter, and finally, the exophthalmic goiter. The results of their analyses are indicated in Table 10. Although glands were classified according to the size of the acini in the gland, no definite correlation was obtained between the size of the follicle and the iodine and thyroxine content. In group 3 of the table are recorded results found with glands obtained at autopsy in which iodine had been painted on the skin for several weeks before death. It will be observed that there was no obvious change due to this treatment. The net result of this study was that thyroglobulin from goiters contains less thyroxine and also less diiodotyrosine iodine than does thyroglobulin from normal human thyroids. Interestingly enough, *as judged from the thyroglobulin*, there is only one type of goiter, and the great difference in types of goiter known to the pathologist must be looked for in some vital phenomenon, but certainly not in the colloid deposited.

In the colloid goiter and nodular goiter group, the basal metabolic rate of the individual from whom the gland was derived was not correlated with the iodine in the gland. In the case of exophthalmic glands, however, the general tendency was for the thyroglobulin to contain less thyroxine if the basal metabolic rate was very high. In general, it is interesting that the iodine content of the thyroglobulin from all types of goiter was usually rather low even when iodine had been administered to the patient. It should be remarked that the thyroxine content of these proteins was estimated by the procedure of Harington and Randall, which tends to give results a little high; that is, when one gets a result of about 35 per cent by this method, one should probably read about 28 per cent. When thyroglobulin from the glands of exophthalmic goiter was analyzed and the results divided into four or five groups, according to the degree of hypertrophy, there was no significant difference in the total iodine percentage of these groups nor in the relative amount of thyroxine iodine, as shown from Table 10. The group numbers, 1 to 4, which head the columns in the table, indicate increasing degrees of hypertrophy as determined by pathological study.

Cavett [80] has extended this analysis of various thyroglobulins by determining the Van Slyke nitrogen distribution due to the various amino-acid constituents of thyroglobulin. A brief summary of his values for normal and pathological glands is given in Table 11.

Similar values were obtained from colloid, adenomatous, or nodular goiters and from exophthalmic glands. The striking result was that, with the exception of thyroxine, diiodotyrosine, and tyrosine, the general analytical picture of all types of thyroid protein was the same. In general, the tyrosine content tended to vary inversely with the thyroxine and diiodotyrosine content. Inasmuch as Harington and Barger long ago suggested that tyrosine was the natural precursor, first of diiodotyrosine and later of thyroxine, it would be pleasant to assume that in the molecule each radical of diiodotyrosine or thyroxine is located at a point where previously a molecule of tyrosine had existed. Accordingly, one would expect in thyroglobulins which are low in iodine that much unconverted tyrosine would remain, and this is seen to be true from the analyses. Furthermore, if the patient has been treated with iodine, one would expect to find higher diiodotyrosine and thyroxine values and correspondingly less tyrosine. The results show that this is also true. Inasmuch as the thyroglobulin from goitrous glands tended to be low in thyroxine-iodine and in diiodotyrosine-iodine, despite the copious use of iodine medication, it might be inferred that the formation of hormone or its precursor was interfered with in these glands. An alternative explanation, however, is that the material, as soon as it acquires hormonal form *in vivo*, is delivered into the general circulation; so that one finds remaining in the glands only material which is in the process of manufacture into hormone.

TABLE 10

THYROGLOBULIN AND DEGREE OF FOLLICULAR HYPERTROPHY

Protein from hypertrophied glands	1	2	2–3	3	4
Total iodine (per cent)	0.31	0.27	0.3	0.269	0.32
Thyroxine iodine (per cent) ...	0.084	0.116	0.115	0.143	0.113

From: J. W. Cavett, C. O. Rice, and J. F. McClendon, J. Biol. Chem. *110*: 679. 1935.

TABLE 11

Average Values Given by Thyroglobulin from Normal and Pathological Glands

	Maximum variations found in normal group	Normal	Colloid	Variation from normal	Adeno-matous	Variation from normal	Exoph-thalmic	Variation from normal
Per cent total N								
Amide N	2.91	9.33	9.82	+0.49	9.93	+0.60	9.81	+0.48
Humin N	1.47	3.93	3.69	−0.24	3.75	−0.18	4.08	+0.15
Arginine N	0.65	14.91	14.75	−0.16	14.81	−0.10	14.90	−0.01
Cystine N	0.60	1.91	2.02	+0.11	1.94	+0.03	1.99	+0.08
Histidine N	0.60	2.99	2.98	−0.01	2.83	−0.16	2.85	−0.14
Lysine N	1.95	6.84	6.45	−0.39	6.65	−0.19	6.73	−0.11
Filtrate amino N	1.41	56.77	56.49	−0.28	56.02	−0.75	55.69	−1.08
Filtrate non-amino N	1.87	4.90	4.80	−0.10	5.42	+0.52	5.38	+0.48
Per cent total protein								
Tryptophane	0.26	2.13	2.15	+0.02	2.14	+0.01	2.13	0.00
Tyrosine	0.15	3.29	3.38	+0.09	3.23	−0.06	3.09	−0.20
Tyrosine + tyrosine equivalent	...	3.45	3.40	−0.05	3.39	−0.06	3.35	−0.10
Thyroxine	...	0.32	0.02	−0.30	0.10	−0.22	0.19	−0.13
Diiodotyrosine	...	0.277	0.05	−0.227	0.31	+0.217	0.49	+0.213

From: J. W. Cavett, J. Biol. Chem. *114*: 71. 1936.

ARTIFICIAL IODOPROTEINS

Preparation. There are three general methods for iodinating proteins, namely, in acid, in alkaline, and in neutral media. In all three the end result differs as certain conditions are modified, e.g., the temperature, the iodine carrier, and the concentration of the iodinating medium. Each method has certain advantages and disadvantages which influence the choice thereof. Hofmeister [248] practiced iodination in an acid medium with elementary iodine at room temperature. He obtained characteristic values for the "iodine number" of various proteins, but Blum [50] showed that these values tended to be lower than the values obtained in media made alkaline by carbonate. Blum and Strauss [48] later compared these two media and concluded that the acid led to splitting of the protein molecule. In consequence of this hydrolysis, an extra quantity of iodine united with the split products and led to a spurious increase in the (acidic) iodine number. Nevertheless, the acid method has certain advantages in special cases. For example, Bauer and Strauss [38] have used this procedure to show that iodine could enter the histidine radicals in globin. Indeed, by varying the concentration of acid, they obtained several different iodine numbers for separate varieties of globin and their derivatives, as described later.

The bicarbonate procedure used by Blum and his collaborators has been employed extensively with various modifications. In the last century Blum and Vaubel [51] found that after iodinating egg albumin the resulting material contained only 4 per cent of iodine, whereas if the protein were purified and re-iodinated several times, nearly 7 per cent could be attained. They showed, further, that hydrogen iodide accumulated and prevented the reaction from running to completion. Blum therefore added sodium bicarbonate to combine with the hydriodic acid and the reaction then proceeded to completion.

Afterwards, Blum and Strauss [49] found that when proteins were treated with iodine for several hours in the presence of bicarbonate, three secondary changes took place in the protein molecule: (*a*) oxidation of the tryptophane groups; (*b*) oxidation of the cystine residues; and (*c*) destruction of one-half of the groupings in the protein responsible for the biuret reaction. The first two of these side effects could be eliminated by operating for less than ten minutes, i.e., until Millon's reaction became negative. These undesirable, incidental mutilations of the protein molecule led Blum and Strauss to propose the use of ammonium hydroxide as the buffer. Actually, under these circumstances, iodination probably proceeds through the intermediary nitrogen tri-iodide, as utilized [582] by Wormall. The result is an iodoprotein which shows no striking differences from the original substance in so far as physicochemical properties are concerned. In other words, the solubilities, various molecular sizes, and amino-acid constituents show a minimal change from their original state. It is important that when globin is iodinated, the more iodine is bound, the more basic the solution. Indeed, Bauer and Strauss found that when globin was dissolved in twice normal sodium hydroxide and treated with iodine very cold, they obtained an iodoglobin preparation containing more iodine than by any other method. The noticeable effect of alkaline medium with reference to side reactions is of special significance because, as will be pointed out presently, it may have an important bearing upon the development of thyroxine-like activity in such preparations.

Iodination in neutral media has not been used extensively, but it is clear that a considerable amount of iodine can combine with protein at physiological neutrality. For example, Kurajeff [304] used sodium iodide and iodine in neutral solutions at 37°C. for twenty hours. In recent years, Ludwig and Mutzenbecher [344] have introduced 8 per cent of iodine into casein at neutrality by treating for one-half hour at room temperature. These results in neutral medium are of especial interest from a physiological standpoint, because it is now known that such preparations can yield pure thyroxine after appropriate subsequent treatment. Indeed, recently pure crystalline thyroxine has been obtained directly from pure diiodotyrosine.

Physicochemical Peculiarities. On superficial examination, Oswald found that iodinated proteins were very similar to ordinary proteins and he stated that thyroglobulin differed little from any ordinary globulin except in its iodine content. More careful study in recent years, however, has disclosed unmistakable changes after iodination. For example, Cohn [92] has discussed the modification of the dissociating hydroxyl group of tyrosine following the introduction of iodine into the protein molecule. Inasmuch as tyrosine has three pK values, i.e., two due to the dissociating zwitterion and a third due to dissociation of the phenolic group, it is possible to observe the result of introducing two iodine atoms into the molecule. The hydroxyl group then has a stronger dissociation by at least a thousandfold and the amino group also dissociates at a somewhat more acid reaction, as shown by the values in Table 12. It should be noted carefully that the numbers of the dissociating groups shown in the table refer only to tyrosine, and are arranged in the order of decreasing acidity. The values for diiodotyrosine, contrary to the usual convention, are listed out of order so as to emphasize the relative inversion of hydroxyl and amino groups as regards their comparative acidic strength. This exchange is due largely to the greatly increased acidic nature of the hydroxyl group.

TABLE 12

COMPARISON OF THE pK VALUES OF TYROSINE AND IODOTYROSINE *

		$pK_1(COOH)$	$pK_2(NH_2)$	$pK_3(OH)$
Tyrosine	...	2.24	9.21	10.28
Diiodotyrosine	0°	2.21	8.31	6.53
	25°	2.12	7.82	6.48
	40°	2.13	7.45	6.45

* The reader will please note that the traditional numbers for the respective groups of diiodotyrosine have been falsified deliberately, as stated in the text.

From: E. J. Cohn, Ergebn. d. Physiol. *33*: 846. 1931.

If these values, obtained with free tyrosine and diiodotyrosine, apply also when these amino-acids are incorporated into proteins, it should be possible to detect a predictable difference between a given protein and the homologous iodoprotein. Neuberger [407] has actually made this test in the case of zein. He iodinated the protein by the ammonia method with a compound solution of iodine in potassium iodide. He used 5 per cent more iodine than the amount calculated as necessary to supply each tyrosine radical with two iodine atoms. His product contained 7.54 per cent of iodine as compared with the theoretical value of 7.65 per cent, and he concluded, therefore, that the iodine had entered exclusively into tyrosine groups. Further evidence of this was the absence of a Millon's reaction and the presence of an intensively positive test for the ortho-diiodophenolic grouping after treatment with nitrous acid and ammonia as described by Kendall and Osterberg.[277] This iodozein closely resembled pure zein in its solubilities; that is, it was insoluble in organic solvents including pure alcohol, and also insoluble in neutral or acid aqueous solutions. In aqueous alkali, however, it was soluble at a much lower pH value than pure zein.

Neuberger titrated solutions of the two proteins in 90 per cent alcohol, comparing their respective acid-binding and base-binding capacities at different pH values as determined by electrometric titration. These results he compared with the values to be expected from the known composition of pure zein, based on its amino-acid constituents. His experimental values checked closely with the theoretically calculated values within the limit of experimental accuracy, as shown in Table 13.

TABLE 13

Acid-Binding and Base-Binding Capacities of Zein and Iodozein

	Experimental		Calculated	
	(*All figures in moles × 10^{-5} per gram protein*)			
	Acid	*Base*	*Acid*	*Base*
Zein	19.3	54.2	14.2–16.2	60.0
	16.8	46.2		
	20.1	54.6		
	16.6	47.2		
	Av. 18.2	50.6		
	Total	69		
Iodozein	16.8	46.2		
	16.2	48.5		
	Av. 16.5	47.4		
	Total	64		

After: A. Neuberger, Biochem. J. *28*: 1986 and 1990. 1934.

Having shown the approximate stoechiometric identity of the two proteins as regards their total dissociation, Neuberger proceeded to compare the range of acidity in which the tyrosine groups reacted. As shown in Figure 3, most of the base binding is due to the tyrosine residues which react in a strongly alkaline range, i.e., from about pH 11 to pH 13. In both proteins, the number of tyrosine groups was estimated at 41 to 44, and the experimental values found were 42 to 43. As shown by the titration curve for the iodozein, nevertheless, this artificial protein dissolved at a much lower pH range, and this range is precisely the region to be predicted from the pK values for iodotyrosine as listed above! In conclusion, then, Neuberger's iodozein behaved exactly as the properties of the constituent tyrosine groups led him to predict.

That all proteins do not react in so clear-cut a fashion was shown by the results of Haurowitz[231] and of Cohn, Salter, and Ferry.[93] The latter study consisted in an experiment on globin very similar to Neuberger's study of iodozein. Comparative electrometric titration curves from pH 1.6 to 12.5 were obtained for globin and iodoglobin, respectively. The acid-binding capacity of globin was 138×10^{-5} mols per gram and the base-binding capacity was 89×10^{-5} mols per gram, a total dissociation of about 225×10^{-5} mols per gram over the entire range of the pH scale. Of this total, the base combined from pH 7.5 to 12.5 was assigned to lysine, arginine, and tyrosine; that from pH 5.0 to 7.5 to histidine; and the acid combination from pH 1.6 to pH 5.0 to carboxyl groups. When an iodoglobin was prepared by incorporating exactly enough iodine to combine with the tyrosine residues in the protein, the resulting iodoglobin failed to change, as did Neuberger's iodozein. To be sure, the acid-combining capacity was unchanged, but the base bound at pH 12.5 was decreased by an amount approximately equivalent to the number of mols of iodine combined with the globin. These findings suggested that in this iodoglobin the diiodotyrosine mole-

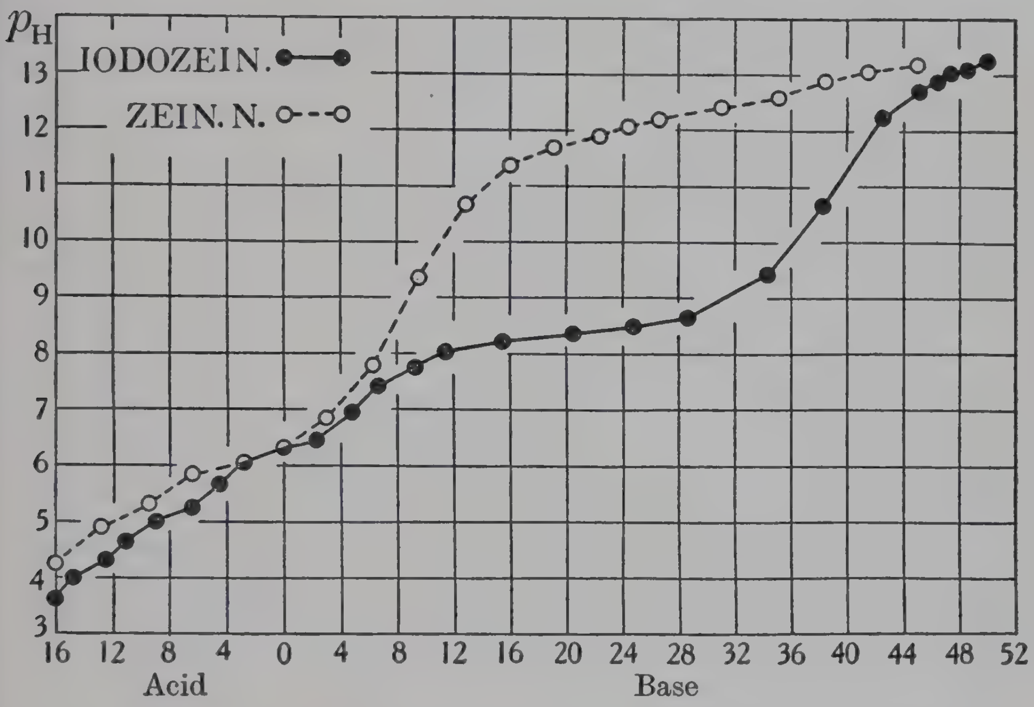


FIG. 3. Comparative titration curves of zein and iodozein. The units indicated by the figures in the abscissa for acid or base are mols $\times 10^{-5}$ per gram of protein.
From: A. Neuberger, Biochem. J. *28*: 1990. 1934.

cules were unable to dissociate as they did in iodozein. In so far as the titration curve was concerned, therefore, the hydroxyl groups of the iodinated tyrosine had apparently disappeared. When larger amounts of iodine were introduced into the globin molecule, the base binding was increased and the acid binding decreased; nevertheless, the total dissociation from pH 1.6 to 12.5 remained at 222 × 10^{-5} mols per gram. In other words, once the tyrosine groups had been iodinated, further iodine in the molecule failed to alter this total value.

This apparent absence of the tyrosine shift observed by Neuberger is of particular interest from the standpoint of the origin of thyroxine from iodinated proteins containing tyrosine. This latter reaction at present mystifies some of the world's best chemists, who recognize no synthetic mechanism which would yield thyroxine directly from diiodotyrosine.[225] The results cited above suggest that the hydroxyl groups in an iodinated protein may become blocked, and this finding might conceivably explain why an iodinated protein can relieve human myxedema when fed directly to patients without further chemical treatment. The question of the origin of endocrine activity in iodoproteins will be discussed in detail in Chapter V.

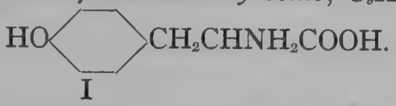
Another peculiarity of iodoglobin was described by Haurowitz,[231] who found that treatment with iodine prevented the coupling of hemin with globin. Ten equivalents of iodine sufficed to destroy this coupling reaction completely, but the mechanism by which the effect was produced is not yet clear.[305]

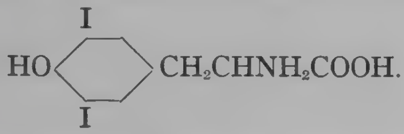
The Iodine-containing Amino-Acids of Iodoproteins

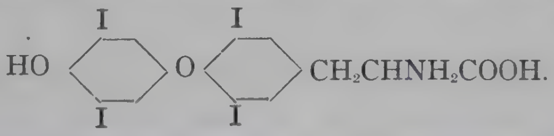
Thus far, the presence of five iodo-amino-acids is suspected in iodoproteins. Of these, three have been isolated directly, i.e., monoiodotyrosine, diiodotyrosine and thyroxine. In addition, monoiodohistidine has been prepared in the form of an anhydride or a histidine derivative. Diiodohistidine remains suspect.

The isolation of diiodotyrosine, first as iodogorgonic acid by Drechsel,[126] and its identification by Wheeler and Jamieson (1905)[574] have been described in detail by Harington, who, with Randall, first isolated the compound from thyroid tissue. These latter investigators,[228] using enzymic digests provided by Salter in Harington's laboratory, were able ultimately to obtain a crystalline compound identical with synthetic d-3:5 diiodo-l-tyrosine. Racemic preparations of the diiodotyrosine had been obtained early in this century by Oswald [421] also from iodo-albumin, from iodocasein, and from gorgonins and sponges. In 1915, Kendall isolated racemized thyroxine from mammalian thyroid tissue;[272] in 1931 the isolation of natural l-thyroxine from the thyroglobulin which had been split by enzymes was described by Harington and Salter [229] and later confirmed by Foster and collaborators.[177] In recent years Ludwig and Mutzenbecher have isolated the same product in its racemic form from iodocasein, as confirmed by Harington.[225] The German authors report also the isolation of mono-iodotyrosine from such digests.

The properties of these amino-acids may be summarized as follows:

Chemical and Physical Properties of Monoiodotyrosine, $C_9H_{10}O_3NI \cdot H_2O$. The formula of monoiodotyrosine is  $HO\underset{I}{\langle\quad\rangle}CH_2CHNH_2COOH$. It contains 39.1 per cent of iodine and 4.3 per cent of nitrogen in the crystalline form which contains one molecule of water of crystallization. It separates out of weakly acidic aqueous solution in the same crystalline form as diiodotyrosine, forming flat whetstone-shaped needles which also form star-shaped clusters. It melts with decomposition at 203° to 207° C. It dissolves in four normal hydrochloric acid on heating, and on cooling the hydrochloride crystallizes in the form of needles. It gives the characteristic color reaction of Kendall and Osterberg with nitrous acid.[344]

Chemical and Physical Properties of Diiodotyrosine. The formula of diiodotyrosine is  $HO\overset{I}{\underset{I}{\langle\quad\rangle}}CH_2CHNH_2COOH$. It contains 58.7 per cent iodine and 3.2 per cent nitrogen. It occurs naturally as 3,5-diiodo-l-tyrosine. It crystallizes in white needles which melt at 199° C. Its solubility in water at 15° C. is about 0.3 per cent. It can be made synthetically by iodinating l-tyrosine.[574]

Chemical and Physical Properties of Thyroxine. The chemical formula of thyroxine is $C_{15}H_{11}O_4NI_4$. It contains 65.4 per cent iodine and 1.8 per cent nitrogen. The atoms are distributed thus:  $HO\overset{I}{\underset{I}{\langle\quad\rangle}}O\overset{I}{\underset{I}{\langle\quad\rangle}}CH_2CHNH_2COOH$. Therefore, technically its precise chemical name is l-β-[3,5-diiodo-4 (3′5′ diiodo-4′ hydroxyphenoxy)-phenyl]-α amino-proprionic acid. It is isolated as a colorless, odorless microcrystalline powder which is very insoluble in water at physiological reactions. On rapid heating, it sinters at from 230° to 235° C. When precipitated from mildly acidic alcohol it forms sheaves or rosettes of crystals. It forms mono- and di-sodium salts, likewise mono- and di-potassium salts, silver salts and acetyl derivatives.[24] The form made from natural thyroglobulin by the action of proteolytic enzymes is levorotatory.[229, 179] Thyroxine can be dissolved

TECHNOLOGICAL RESEARCH
LIBRARY

safely in cold dilute potassium or sodium hydroxide (up to 5 N) and reprecipitated by acidification. The di-sodium salt is quite easily soluble in water, a point of importance from the standpoint of therapy.[544]

These two compounds give a characteristic cerise color with nitrous acid under suitable conditions. This color reaction of Kendall and Osterberg [277] is given not only by thyroxine and by diiodotyrosine, but also by other benzene derivatives which contain two iodine atoms in the ortho positions to a hydroxyl group.[223]

Presumably alkaline digests of iodoproteins may contain other organic iodine compounds, possibly diiodothyronine derivatives or degradation products of thyroxine. Indeed, Salter [471] isolated from baryta-hydrolysates of iodinated serum protein a crystalline compound with properties similar to thyroxine, but (*a*) containing no nitrogen, and (*b*) giving no ammoniacal nitrous acid reaction. Its proximate analysis was as follows: carbon 24 per cent, hydrogen 1.5 per cent, iodine 65 per cent, nitrogen 0.3 to 0.6 per cent. The corresponding values for thyroxine are: carbon 23 per cent, hydrogen 1.4 per cent, iodine 65 per cent, nitrogen 1.8 per cent. Further studies of these alkaline digests of iodoproteins are urgently needed because the isolation of chemical intermediates between diiodotyrosine and thyroxine may furnish important clues as to the mechanism whereby thyroxine arises in the animal body.

In this connection the results of Barkan and Kingisepp [30] on the tryptic digestion of iodinated albumin may indicate a possible explanation of the conversion of "D" into "T" iodine. These authors digested "jodtropon" with trypsin and differentiated two resulting chemical fractions containing organically bound iodine. The first of these fractions was precipitable in acid solution with silver salts; the second fraction yielded no such precipitate. More important was the finding that the two fractions were in equilibrium and mutually interconvertible.

Other Possible Iodine-binding Amino-acids. It has been stated as a fact, known for many decades, that proteins bind varying amounts of iodine, depending upon the procedure and the amounts used. Thus Liebrecht (1897)[325] obtained from casein three preparations, namely: (*a*) "iodocasein" with 5.7 per cent iodine, (*b*) "casejodin" with about 8.7 per cent iodine, and (*c*) "perjodcasein" with 17.8 per cent iodine. He found that these preparations had somewhat different properties, but perhaps his most interesting finding was the interrelation of these three iodine components. He noted that *a* plus *b* approximately equalled *c*, as far as iodine percentages were concerned. He suspected, therefore, that the iodine entered the molecule in successive stages, being bound by different chemical groupings. As proof of this hypothesis, he showed that treatment of preparation "*c*" with sodium bisulfite removed the major part of the iodine to yield preparation "*a*." In 1898, Hofmeister treated crystalline egg albumin with sulfuric acid and hydrogen iodide, and obtained an iodoalbumin containing 8.1 per cent of iodine. About the same time Blum and Vaubel iodinated a number of proteins and demonstrated that when standard procedures were used each pure protein combined with a characteristic amount of iodine. Thereupon began a long series of researches, which are still incomplete, for the purpose of determining the sites of combination within the molecules of these various bound iodine atoms.[238]

Early in this century, for example, Oswald began to hydrolyze iodoproteins in an attempt to isolate the various participating iodo-amino-acids. He iodinated the split products of proteins and found that by this procedure he could obtain impure organic preparations with iodine content well over 20 per cent. He also tested the hypothesis that only tyrosine radicals combined with iodine, by comparing the reactivity of casein and glutin. Casein is rich in tyrosine and gives a strong Millon's reaction, whereas glutin contains but little tyrosine and consequently shows only a faint Millon's test under comparable circumstances. Oswald found, indeed, that casein could add iodine up to 13 per cent, whereas glutin added no more than 2 per cent. This fact suggested strongly that iodocasein owes much of its iodine to diiodotyrosine. Nevertheless, since glutin was able to add a very definite moiety of iodine, Oswald suspected that groups other than tyrosine must be involved in the binding of iodine. He tested his hypothesis further by iodinating two fractions of hydrolyzed albumin which Pick [437] had described as proto-albumose and hetero-albumose. These two preparations had very similar properties and bound iodine in about the same proportion, namely 12.3 per cent and 10.3 per cent, respectively. As seen in Table 14, however, they differed considerably in their constituent amino-acids.

Thus, the high iodine content of the iodinated hetero-albumose could not be accounted for by tyrosine and therefore one or more additional amino-acids must participate in the union.

This hypothesis was advanced on a quantitative basis by Blum and his scholastic descendants, first by Strauss and later by Bauer. Phenylalanine and tryptophane were eliminated from the list of possible chemical groupings; and when Pauly discovered (1910)[427] that the iminazol ring could add iodine, the theoretical background of the problem advanced rapidly. It was found that the more basic the iodinating medium the more iodine could be added; maximal iodination yielded a tetraiodide. It was shown also that the iodine atom which was bound to nitrogen could be removed easily. Although it proved impossible to iodinate free histidine, it was possible to prepare tetraiodohistidine anhydride and para nitrobenzoyl diiodohistidine which served as structural analogs.

TABLE 14

MODES OF LINKAGE OF IODINE TO PROTEIN

PROTO-ALBUMOSE	HETERO-ALBUMOSE
Few di-amino acids	Many di-amino acids
Little or no leucine	Much leucine
No glycocoll	Much glycocoll
Many mono-amino acids	Few mono-amino acids
Much tyrosine	Little tyrosine
Many indol complexes	Few indol groups
Easily split in tryptic digestion	Difficult to split with trypsin
	Much phenylalanine

After: E. P. Pick, Ztschr. f. physiol. Chem. *28*: 219. 1899.

TABLE 15

IODINE NUMBERS OF SOME ARTIFICIAL IODOPROTEINS

	A-value	B-value	C-value	*N*–I/*C*–I
Iodo-ovalbumin	7.55	5.12	4.91	1:2
Iodoserum albumin	8.96	6.73	6.7	1:3
Iodoserum globulin	8.3	6.64	...	1:4
Iodothyroglobulin	6.14	4.88	4.96	1:4
Iodocasein	7.51	7.51	7.51	0:2?

After: F. Blum and E. Strauss, Ztschr. f. physiol. Chem. *112*: 164. 1921.

Blum and Strauss[48] (1920) treated iodinated proteins with sulfurous acid and, with the exception of iodocasein, were able to remove part of the iodine in each case. They named this moiety N-iodine, in contradistinction to the C-iodine fraction which was tightly bound by tyrosine *in carbon linkage*. As shown in Table 15, the ratio of N-iodine to C-iodine for any pure protein was constant and characteristic. The maximal iodination value they called the "A" value; when such substances were treated with sulfurous acid the nitrogen-bound iodine was removed to yield "B"-substances. As shown in the table, these B-substances coincided extraordinarily well with the C-substances obtained by direct iodination, thus corroborating the pioneer work of Liebrecht [325] published a generation earlier.

These various results suggested that in the B-substances iodine existed only in carbon linkage (kernjod). This type of linkage might include diiodotyrosine as well as iodinated histidine. As far as histidine was concerned, the method of combination remained mysterious until 1936, when Bauer and Strauss [37] elucidated the problem from the results of careful quantitative analyses of the constituent amino-acids of proteins. These data have led to a working conception of the mechanism of step-by-step iodination of proteins, which, although still tentative, serves to clarify the various facts pertinent to the problem. The main points in this general conception of iodination may be summarized as follows: Experimentally, it is possible to attain all the theoretical iodine numbers of a protein, e.g., globin, ranging from the simple iodination of the two inherent tyrosine groupings (binding 4 iodine atoms) to the complete iodination of both tyrosine and histidine (16 iodine atoms in the case of globin). In cold sodium hydroxide solution the highest iodine numbers were attained for globin, and this fact substantiated Pauly's discovery that histidine binds iodine more readily in basic solution. In other words, histidine could be considered more labile in alkaline medium, and consequently might be iodinated in three arbitrary steps, depicted as follows: (*1*) Iodine combines with the imid group; (*2*) it wanders to one of the two neighboring carbon atoms; (*3*) the released imid group then combines with another iodine atom. The last stage represents the A-value shown in the table, and this last iodine can be removed by treatment with sulfurous acid.

It is impossible to review in detail here all of the results published by Blum's academic family,[36] but a few striking features can be mentioned. Whereas the iodine numbers of egg albumin, serum albumin, and serum globulin could be explained satisfactorily by conversion of their inherent tyrosine to diiodotyrosine, on the other hand globin could add much more iodine, presumably because diiodohistidine was formed. From nitroglobin an iodoprotein was obtained which still retained one nitro group in the tyrosine ring; its iodine number was only three-quarters that of completely iodinated globin. This value (5.8 per cent iodine) was stoechiometrically equal to the histidine present (7.6 per cent). Thus one obtained a depleted A-substance in which no tyrosine was substituted with iodine. As further proof of this hypothesis, it was found[38] that neither the A-substance nor this depleted A (nitro-) compound could be digested by pepsin. Nevertheless, since both of these A-substances contained nitrogen-bound iodine, it was predicted that sulfurous acid would remove this latter iodine, and that *then* the resulting compounds would be split by pepsin. This was actually found to be the case! In short, the chemists' picture of the iodination of globin involved (*a*) initial iodination of tyrosine, followed by (*b*) iodination of histidine (carbon linkage through nitrogen), followed by (*c*) iodination of the liberated NH group of histidine. This progressive iodination of histidine is illustrated by the theoretically computed values for iodine content shown in Table 16 which, as stated above, Bauer and Strauss have in fact been able to duplicate experimentally.

TABLE 16

THE THEORETICAL STEPWISE ADDITION OF IODINE TO GLOBIN

					Computed Iodine %	No. of I atoms added	Notes
101.08 g.	iodoglob.	contain	1.086 g.	iodine	1.07	1	
102.16 g	"	"	2.173 g	"	2.13	2	
103.23 g	"	"	3.259 g	"	3.16	3	Tyrosine no.
104.31 g	"	"	4.345 g	"	4.17	4	
105.34 g	"	"	5.382 g	"	5.11	5	
106.37 g	"	"	6.418 g	"	6.03	6	
107.39 g	"	"	7.455 g	"	6.94	7	
108.42 g	"	"	8.491 g	"	7.83	8	
109.45 g	"	"	9.528 g	"	8.71	9	Highest C-Iod. no. ("B"-value)
110.48 g	"	"	10.564 g	"	9.56	10	
111.51 g	"	"	11.601 g	"	10.40	11	
112.54 g	"	"	12.637 g	"	11.23	12	
113.56 g	"	"	13.674 g	"	12.04	13	
114.59 g	"	"	14.710 g	"	12.84	14	
115.62 g	"	"	15.747 g	"	13.62	15	Max. I no. (C–I+N–I) ("A"-value)
116.65 g	"	"	16.783 g	"	14.39	16	

From: H. Bauer and E. Strauss, Biochem. Ztschr. *284*: 200. 1936.

This picture advanced by Bauer and Strauss is by no means complete. For example, it does not explain why all known proteins except globin can add iodine only to the imid group of histidine. It does not explain, further, why histidine adds only one iodine atom to carbon. Possibly the answer to these questions involves the properties of zwitterions formed by the histidine in protein combination, but this remains a mere suggestion. At any rate, this careful quantitative work has laid down a rational basis for the formation of iodinated albuminous compounds, which have been shown to have profound endocrinological effects and immunological properties.[582]

SPLIT PRODUCTS OF IODOPROTEIN

If one starts with an iodoprotein made from mammalian blood serum, or from partially purified serum albumin solution which has been iodinated with compound solution of iodine according to the procedure of Wormall,[582] one obtains within a short time an iodoprotein which may contain more than 18 per cent of iodine. The protein can be separated and purified by repeated precipitation at its isoelectric point near pH 5.0. This technique has been described at length by Lerman and Salter.[321] One thus obtains from a natural circulating protein, by a very simple chemical procedure, an iodoprotein which bears many resemblances to natural thyroglobulin, although the similarities are by no means complete. This material can be separated into "T" and "D" fractions; indeed, this separation may be achieved either by acid hydrolysis, by alkaline hydrolysis or through the action of proteolytic enzymes. As shown in Figure 4, by using pepsin, trypsin and alkali successively, one can split down the protein molecule in stages, so that one prepares first a "T" peptone and a "D" peptone, then a "T" peptide and a "D" peptide fraction, and, ultimately, one arrives at mixtures of "T" amino-acids and "D" amino-acids. In all these cases the "T" fraction shows minimal solubility near pH 5.0, whereas the "D" peptone is relatively soluble over the entire range of acidity. The precise biological activity of these fractions will be discussed in a later chapter, but, in general, endocrine activity similar to thyroid hormone remains in the "T" fraction at each of these successive stages. Indeed, from iodocasein Ludwig and Mutzenbecher [344] have finally isolated crystalline thyroxine. As this fractionation proceeds, the iodine in the "D" fraction does not change significantly until the peptide level is reached. In the amino-acid level, however, even crude mixtures of "T" material contain 25 per cent of iodine, and on simple purification, such as extraction with alcohol or acetone, this percentage rapidly increases to over 40 per cent.

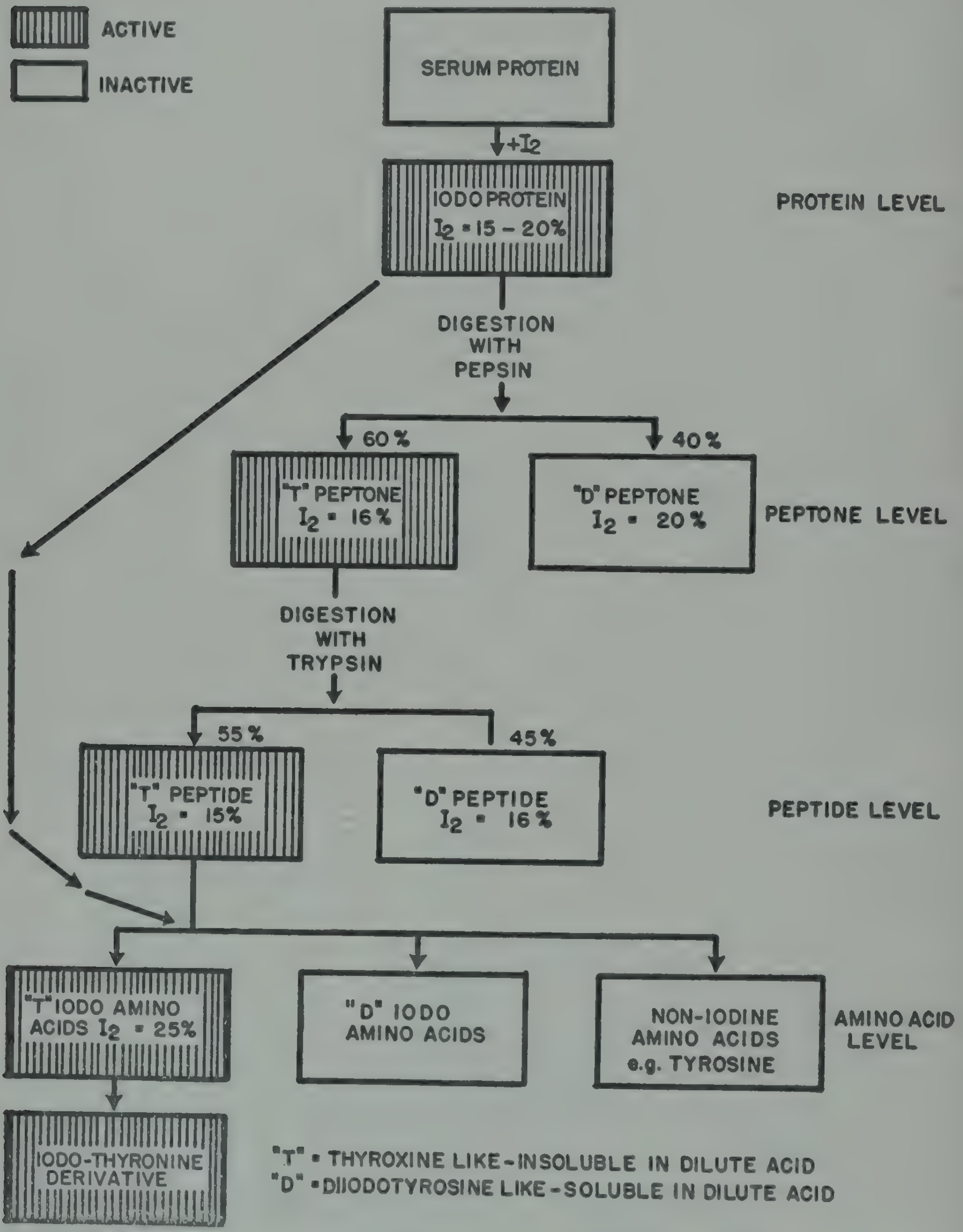

ACTIVE
INACTIVE
SERUM PROTEIN
+I2
IODO PROTEIN I2 = 15 – 20%
PROTEIN LEVEL
DIGESTION WITH PEPSIN
60%
40%
"T" PEPTONE I2 = 16%
"D" PEPTONE I2 = 20%
PEPTONE LEVEL
DIGESTION WITH TRYPSIN
55%
45%
"T" PEPTIDE I2 = 15%
"D" PEPTIDE I2 = 16%
PEPTIDE LEVEL
"T" IODO AMINO ACIDS I2 = 25%
"D" IODO AMINO ACIDS
NON-IODINE AMINO ACIDS e.g. TYROSINE
AMINO ACID LEVEL
IODO-THYRONINE DERIVATIVE
"T" = THYROXINE LIKE-INSOLUBLE IN DILUTE ACID
"D" = DIIODOTYROSINE LIKE-SOLUBLE IN DILUTE ACID

FIG. 4. Diagrammatic representation of the formation of iodoprotein and its hydrolysis to peptones, peptides and amino-acids.

From: J. Lerman and W. T. Salter, Endocrinology, *25*: 714, 1939.

If one compares the behavior of this iodinated blood protein with that of natural thyroglobulin, as fractionated by Harington and Salter,[229] one notes at once that the difference between the two protocols has chiefly to do with the much higher percentages of iodine involved in the case of the serum protein. In other words, the nitrogen: iodine ratios for the split products of the iodo-albumin are much lower than for thyroglobulin. This same discrepancy holds also for the pharmaceutical activity of the preparations when tested in athyreotic rabbits or in clinical cases of human myxedema. Nevertheless, the response of the human myxedema syndrome to this artificial iodoprotein is complete and classical in every respect. Indeed, recently Lerman and Salter [321] have observed the induction of an Addison's crisis in a patient whose inadequate adrenal cortical function was completely masked by the obvious picture of myxedema. This effect is exceedingly characteristic of natural thyroid medication (cf. Chapters VI and XI), and indicates simply that this iodo-albumin is able to produce nearly lethal endocrine imbalance under precisely the same circumstances that natural thyroid does. Consequently, it is clear that the artificial protein may be considered, qualitatively at least, as a bona fide preparation of the thyroid hormone.[79] (Cf. J. H. Means, Tr. Assn. Am. Phys., 1940.)

In fact, when the iodo-albumin is split into small fragments the discrepancy in activity between it and natural thyroglobulin tends to become smaller. Because of the large amount of inert iodine originally introduced into the serum protein, the activity of the iodoprotein is only about one four-hundredth that of whole thyroid in terms of iodine content, but even at this stage, in terms of total mass, the iodoprotein is already about one-fifth as active as natural thyroglobulin. After the elimination of the "D" peptone the first ratio becomes about 1 to 200 and, in terms of mass, the acid-insoluble peptone becomes about half as active as natural thyroglobulin. Further hydrolysis with baryta, a procedure similar to the standard method for obtaining thyroxine from thyroglobulin, finally yields a product which is about one thirty-fifth as active as thyroglobulin in terms of iodine or, in terms of mass, about as active as crystalline diiodothyronine, namely, about 5 per cent as active as thyroxine. In Fig. 5 are illustrated characteristic responses in human myxedema.

Inasmuch as material of this sort can be made at physiologically normal pH values, within a very few minutes *in vitro*, the suggestion at once arises that some such process occurs in the gland itself under enzymic influence. This thought leads to several subsidiary problems: (*a*) does thyronine exist preformed in serum protein as an essential amino-acid, awaiting iodination? (*b*) does the process of iodinating the protein also change molecular configuration so as to produce physiological activity? (*c*) can the thyroidless organism synthesize iodothyronine molecules from other iodinated residues? [320] Harington [225] has recently pointed out that the genesis of thyroid activity under the conditions just mentioned is extremely mystifying and this problem has already aroused a good deal of speculation from the leading biochemists of our day. As a physiological by-product of this discussion, there arises the possibility also that the thyroid gland

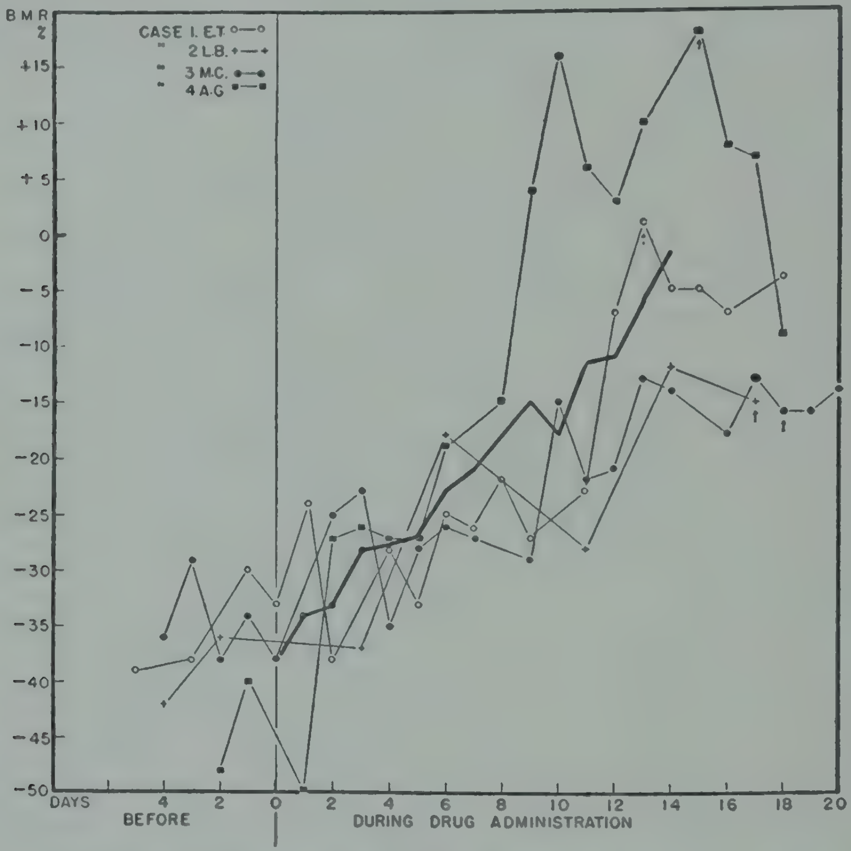


FIG. 5. Metabolic response of three patients with myxedema and one with cretinism receiving an alkaline (baryta) hydrolysate of iodinated serum protein. The heavy solid line is a standard curve of reference representing the metabolic response to the daily administration of thyroxine polypeptide containing 0.5 mg. of iodine.

From: W. T. Salter and J. Lerman, Trans. Assn. Am. Phys. *53*: 204. 1938.

does not manufacture hormone *de novo*, but that it merely concentrates and stores iodinated materials which are already pre-existent in the circulating blood. At any rate, these observations are distinctive in bridging the gap between nutrition and endocrinology by a mechanism which can be described in terms of familiar classical chemistry. (Cf. P. Block, Jr., 1940.)

Peptones and Plasteins of Thyroglobulin. As shown by Harington and Salter,[229] it is possible to split down natural thyroglobulin into active and inactive "T" and "D" proteolytic fragments; the endocrine activity remains largely with the "T" moiety in each case. Salter and Lerman [470] have pursued this partition further by digesting natural human thyroglobulin with pepsin. As shown in Fig. 6, the natural iodoprotein yields a diiodotyrosine peptone which remains soluble at pH 5.0. This material is small enough to pass through a cellophane membrane (No. 300 plain). By this ultrafiltration (under pressure) unsplit protein impurity is filtered out

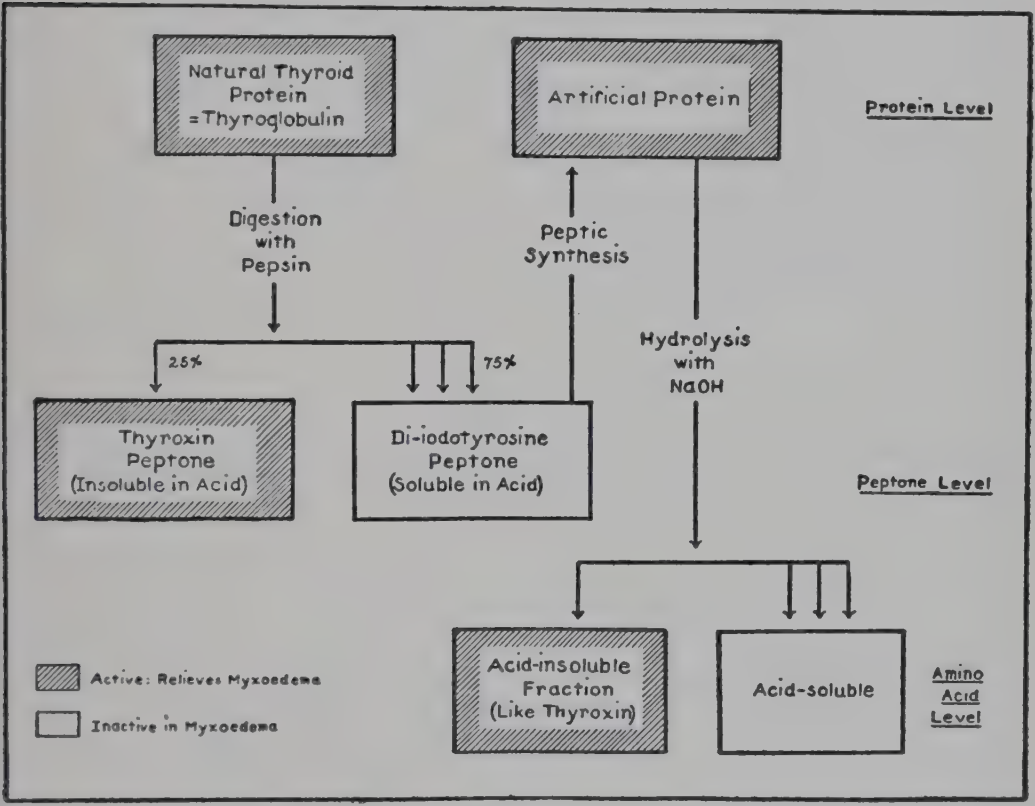

Natural Thyroid Protein =Thyroglobulin
Artificial Protein
Protein Level
Digestion with Pepsin
Peptic Synthesis
Hydrolysis with NaOH
25%
75%
Thyroxin Peptone (Insoluble in Acid)
Di-iodotyrosine Peptone (Soluble in Acid)
Peptone Level
Active: Relieves Myxoedema
Inactive in Myxoedema
Acid-insoluble Fraction (Like Thyroxin)
Acid-soluble
Amino Acid Level

FIG. 6. Diagrammatic representation of the relationships between natural and artificial thyroid protein and their respective cleavage products.

From: W. T. Salter and J. Lerman, Endocrinology, *20*: 802. 1936.

and, with it, also impurities belonging in the "T" fraction which are held back by "adsorption" on the retained colloid. In this way one obtains a solution of diiodotyrosine peptone which is inert when fed to myxedema patients, even in large doses. For example, whereas 0.5 mg. of iodine in the form of natural thyroglobulin produced rapid recovery of the patient, i.e., a standard response in two weeks' time, when five to seven times this dosage was administered in the form of the filtered peptone, Salter and Lerman failed to detect a response in any of three patients so tested. These observations therefore indicated that the diiodotyrosine peptone, when pure, had a negligible effect in human myxedema. On the other hand, it is of technical interest that if the peptone were not filtered first, it contained enough unsplit protein or "T" fraction so that a daily dose of 3.0 to 3.5 mg. of iodine produced a satisfactory recovery in typical human myxedema.

Having thus separated from the natural colloid a large portion of the native protein in peptone form, Salter was able then to recombine the fragments to produce an artificial protein prepared through enzymic synthesis. The method used was suggested many years ago by Danilewski [119] and further studied by Abderhalden in connection with studies on the natural production of body protein. The technique was improved further by Wasteneys and Borsook,[564] who published a rather elaborate mathematical rationale for the effect. A modification of this procedure was used by the author [467] to prepare an artificial thyroid protein or plastein from the purified diiodotyrosine peptone just described. Briefly stated, crystalline pepsin was employed as an enzyme to catalyze the recombination of proteolytic split products, a reaction which is determined by such conditions as concentration, solubility and temperature. The result was an artificial albuminous colloid which contained approximately 0.2 per cent iodine and 14 per cent nitrogen. This material showed many properties in common with the natural thyroid protein. It was precisely as active as the natural product, both in terms of nitrogen or iodine and in terms of total mass. It relieved human myxedema promptly and in characteristic fashion. Furthermore, on treatment with pepsin in strong mineral acid, it again split up into "T" and "D" fractions. The chemical properties of the two proteins, artificial and natural, are compared briefly in Table 17.

Of course, the possible presence of traces of thyroxine impurity in the diiodotyrosine preparation after purification made it essential to control carefully this source of error. This was particularly true because when this work was done most biochemists were extremely skeptical that iodinated nondescript protein could have the properties of thyroglobulin in any degree. Accordingly, Salter and Lerman[470] proposed an arithmetical balance sheet to answer two pertinent questions: (*a*) could the effect of artificial protein represent merely a quantitative precipitation of thyroxine impurity from the solution of diiodotyrosine peptone? and (*b*) could the effect of artificial protein be accounted for by its thyroxine-like fraction alone? For purposes of calculation the ultrafiltered diiodotyrosine peptone described by Salter and Pearson[474] was used. The material was analyzed for apparent thyroxine iodine in two laboratories and values were obtained as follows: in the diiodotyrosine peptone 1 to 2 per cent, and in the artificial protein 12 to 13 per cent. On the basis of these values, the following calculation was made:

TABLE 17

COMPARATIVE PROPERTIES OF ARTIFICIAL THYROID PROTEIN AND NATURAL THYROGLOBULIN

	Artificial protein	Natural protein
Iodine content	0.2 ± per cent	0.2 ± per cent
Nitrogen content	13 per cent	15 per cent
Isoelectric point (denatured)	pH 5.5	pH 5.0
Dialysis	80 + per cent not diffusible	95 + per cent not diffusible
Biuret reaction	++	++
Precipitation by 2 per cent trichloracetic acid	++	++
Digestion by pepsin	++	++
Solubility in 0.01 normal NaOH	++	++
Apparent thyroxine iodine moiety	13–35 per cent	25 ± per cent (Boston)
Molecular weight	18,000 ±	695,000 (Heidelberger and Svedberg)

From: W. T. Salter and J. Lerman, New England J. Med. *216*: 374. 1937.

Calculation I. Granting a 2 per cent thyroxine impurity in the diiodotyrosine peptone, the dose of total iodine required to give a standard 0.5 mg. amount of thyroxine daily would be $\frac{0.5}{0.02} = 25$ mg. From such a 25 mg. portion there was obtained a 15 per cent yield in the form of artificial protein, i.e., 3.75 mg. of iodine. Thus, the actual yield obtained was sufficient to treat $\frac{3.75}{0.5} = 7+$ patients instead of 1. Consequently, mere precipitation of a thyroxine impurity could not account for the observed activity obtained by enzymic synthesis.

Calculation II. The standard daily dose of artificial protein used contained 0.5 mg. of total iodine. This was equivalent to $0.5 \times 0.125 = 0.06$ mg. of thyroxine-like iodine. Accordingly, as stated above, three patients were treated with $\frac{0.06}{0.021} = 3$ mg., or a sixfold daily dose of diiodotyrosine peptone containing 0.06 mg. of possible thyroxine iodine impurity. These patients showed absolutely no relief. Furthermore, when thyroxine peptone itself was resorted to and given in a daily dose of $0.5 \times 0.15 = 0.07$ mg. of iodine to a patient with full-blown myxedema, there was practically no effect or at best a debatable one.

Consequently, the activity of the artificial protein was much greater than its thyroxine-like fraction alone. In a later chapter it will be shown that this is true also of natural thyroglobulin, *when used to treat human myxedema.*

ORGANIC IODINE COMPOUNDS IN RENAL CLEARANCE

In recent years clinical studies of renal clearance have involved the use of organic iodine compounds which originally were used for x-ray purposes. For some years compounds of the sort known as iopax or uroselectan have been used to visualize the renal pelvis and related structures after the absorption of this material into the circulation. The convenience and advantages of this direct pyelography are well known to clinicians. The development of this roentgenological technique ultimately led to the finding that the organic iodine compounds, diodrast and hippuran, have the highest clearances in the human kidney of any known substance. Indeed these clearances are ten times greater than the urea clearance and over five times greater than the inulin clearance.[512] These two substances, together with iopax, neo-iopax, and skiodan, were tested as shown in Table 18. All of them are excreted in part by the renal tubules of the human kidney; all of them depress the phenol clearance as their plasma concentration increases. Apparently all these substances are excreted by a common cellular mechanism, and consequently, they are of great help in the evaluation of renal activity. In addition to being filtered through the glomeruli, they are copiously excreted by the renal tubules. Consequently, when the plasma concentration reaches a point where the excretory mechanism of the renal tubules is functioning at maximum capacity, the rate of tubular excretion can be used to estimate the total active mass of tubular tissue which can still function.

In calculating this maximal rate of excretion, it is necessary to correct for the fraction which is normally excreted by the glomeruli and also for the fraction which is bound to the plasma proteins. When these corrections are made, the excretion of these iodine compounds affords a very convenient method of estimating residual active kidney, and such clearances promise great usefulness in the diagnosis and prognosis of renal disease. Because these substances contain large percentages of iodine, their concentration in blood and urine may be estimated readily with an error of only about 2 per cent. Because the Groâk method,[207] as described in the Appendix, can be used directly with some of these substances without preliminary ashing, the iodine in these substances can be measured easily.

TABLE 18

ORGANIC IODINE COMPOUNDS IN RENAL CLEARANCE

Type of compound		Average inulin clearance ratio	T_m* mM. per minute	Per cent free †	Approximate per cent depression effected by 1.0 mM. per liter in plasma	
					On self-clearance	On simultaneous phenol red clearance
Diodrast	3,5-diiodo-4-pyridone-N-acetic acid	6.0	0.3	72	50	85
Hippuran	Sodium ortho-iodohippurate	5.4	0.6	30	25	80
Iopax	Sodium 2-oxo-5-iodopyridine-N-acetate	5.0	0.2	56	65	75
Neoiopax	Disodium N-methyl-3:5-diiodo-4-pyridoxyl-2:6-dicarboxylate	1.2	0.01	74	25	55
Skiodan	Sodium mono-iodomethane sulphonate	1.2	>0.1	82	±5	±5

* The maximal rate of tubular excretion. The data on diodrast and hippuran are quoted from Smith, Goldring, and Chasis. J. Clin. Investigation, *17*: 263. 1938.

† Per cent of total dye filterable at a total concentration of 1.0 mg. per cent, in human plasma containing 4 per cent albumin.

From: W. W. Smith and H. A. Ranges, Am. J. Physiol., *123*: 723. 1938.

Therefore, they are very convenient for measurement of renal function and yield results of far more fundamental significance than the simple use of iodide as mentioned in Chapter IX.

Apart from their iodine content, several of these substances seem to be completely unrelated, and it is not known at present why they behave so similarly when excreted through the kidney. It seems likely that homologous series of these compounds will be developed for the express purpose of measuring renal function and, in particular, tubular excretory mass in human cases. It will be recalled that these substances and their use are partly an outgrowth of the iodosulfonphenolphthalein test used now for many years in the examination of the gall bladder after the initial researches of Whitaker, and of Graham and Cole.[203] Although the latter types of substances are high in iodine and are of great value because of their opacity to x-rays in visualizing the gall bladder, they do not concern us here from the standpoint of iodine metabolism because direct estimation of their iodine content usually is not used in any well recognized type of physiological or clinical investigation.

CHAPTER IV

CIRCULATING IODINE

IODINE IN RESPIRED AIR

THE atmosphere of lowlands near the sea may contain 0.4 gamma iodine per liter, so that a man conceivably might inhale from 3 to 5 gamma daily. Consequently, it is difficult to estimate iodine truly excreted by respiration. By actual measurement a man can exhale 10 gamma in 24 hours, i.e., 25 per cent of the total excreted.[480] Presumably this iodine is largely in the form of iodide. Szász [534] found as much as 24 to 33 gamma exhaled per day in a mountain atmosphere which was essentially iodine free, but this larger amount may be related to the large water excretion by the lungs at high atmospheres.

IODINE IN CEREBROSPINAL FLUID AND IN LYMPH

The nature of the iodine in cerebrospinal fluid is still unsettled. Its concentration is lower than that of whole blood. The following pairs of comparative values are given in gamma per cent for blood and spinal fluid, respectively: 10.6 and 7.4; [214] 15 and 10; [245] 7.7 and 2.[381] It is likely that the cerebrospinal fluid contains no thyroid hormone,[381] but further study is needed.

Exogenous Iodide in Cerebrospinal Fluid and in Lymph. There are almost no data at present on the influence of doses of iodine small enough to approach physiological conditions. When large therapeutic doses are given, however, there is unquestionably a prompt rise in iodine in the cerebrospinal fluid, although of much smaller magnitude than that in the blood. For example, the administration of half a gram of potassium iodide increases blood iodine to 1424 gamma per cent within two hours, whereas the spinal fluid iodine rises only to 47 gamma per cent.[214] In certain fevers and in carbon monoxide poisoning the concentration may increase by as much as 102 gamma per cent after the administration of iodide. When the drug is forced, i.e., after doses of 4 grams by mouth or 20 grams intravenously, the spinal fluid may contain from 1.8 to 2.8 mg. per cent of iodine.[419] These concentrations are of pharmacological interest only, but they show that iodide may diffuse readily throughout body fluids if the blood concentration is very high.

The lymph contains an appreciable quantity of iodine,[499] but the mechanism which governs its concentration remains obscure. The puzzling fact is that dogs whose *blood* iodine is from 10 to 18 gamma per cent yield thoracic duct *lymph* which contains 42 gamma per cent. Furthermore, when the animals are fed plenty of milk, values of from 290 to 378 gamma per cent are found. Together, these findings suggest that the high concentration in the thoracic duct is due to contributions of iodine from the intestine and the liver.

TABLE 19

Distribution of Iodine and Thiocyanate in Tissue and Blood

	Rat A				Rat B			
	Iodide per gram of dry tissue	Thiocyanate per gram of dry tissue	T/Bl iodide	T/Bl thiocyanate	Iodide per gram of dry tissue	Thiocyanate per gram of dry tissue	T/Bl iodide	T/Bl thiocyanate
	mgm.	mgm.			mgm.	mgm.		
Blood	0.640	3.97	...	...	0.216	2.64	...	...
Liver	0.118	0.950	0.18	0.24	0.058	0.630	0.27	0.24
Muscle ...	0.127	0.920	0.20	0.23	0.042	0.538	0.19	0.20
Skin	....	....	...	...	0.143	1.33	0.66	0.50
Lung	0.224	1.89	0.35	0.48	0.157	1.91	0.73	0.72
Brain	0.085	0.716	0.13	0.18	0.034	0.451	0.16	0.17
Spleen	0.204	1.38	0.32	0.35	0.095	1.35	0.44	0.51

From: G. B. Wallace and B. B. Brodie, J. Pharmacol. and Exper. Therap. *61*: 405. 1937.

As to the peripheral lymph, there is a dearth of direct evidence. Indirect evidence is available, however, in studies of the distribution of administered iodide and thiocyanate in comparison with chloride. These data have been reviewed by Wallace and Brodie [563] and supplemented with analyses of blood, of serum and of cystic and transudate fluids. In general, the relative concentration of the three anions is similar, *provided* the results are expressed in terms of appropriate ratios, i.e., tissue/blood or tissue/serum.* Consequently iodides, like chlorides, are probably distributed mostly in extracellular fluid as described by J. P. Peters in "Body Water." †

---

* A notable exception is the brain, in which chloride occurs in much larger relative amounts.

† When an organic compound containing iodine is administered, some of the iodine is converted into iodide and a mixed picture results, as described by Barkan and Leistner (Klin. Wchnschr. *8*: 117. 1929).

Obviously, the extracellular water must normally also contain thyroid hormone, but it has not been measured directly. As shown in Tables 19 and 20 the relative concentration of these three ions was the same except for the brain, which accumulated much more chloride proportionately. Accordingly, it seems likely that most of the circulating iodide is distributed in extracellular fluid, just as chloride is. Of course, this statement applies only to concentrations of iodide such as are reached after therapy with preparations like Lugol's solution. In the vanishing physiological concentrations, obviously some organic iodine must permeate body cells and extracellular fluids in the form of thyroid hormone. The similarity of distribution, however, of iodide, chloride and thiocyanate makes a selective chemical or physical relationship of iodide to tissue cells improbable. Similarly, even in transudates or cystic fluids which occur in pathological conditions, iodides, like thiocyanates, are distributed in a manner similar to chloride.

TABLE 20

CALCULATION OF BODY FLUID OF VARIOUS ORGANS IN WHICH IODIDE AND CHLORIDE ARE DISTRIBUTED

		Iodide per gram of wet tissue	Chloride per gram of wet tissue	PER CENT INTERSTITIAL FLUID CALCULATED FROM	
				Iodide	Chloride
		mgm.	mgm.		
Serum		0.184	3.82	..	..
Liver		0.050	1.14	27	30
Muscle		0.031	0.49	17	13
Skin		0.142	2.40	77	63
Lung		0.119	2.42	65	63
Kidney		0.119	2.35	65	61
Stomach		0.097	2.00	53	52
Pancreas		0.064	1.45	35	38
Spleen		0.064	1.54	35	40
Adrenals		0.062	1.40	34	37
		Per gram of dried blood		Ratio of dried blood to serum	
Cat	Blood	4.26	19.5	4.3	4.3
	Serum (per cc.)	0.978	4.47		
Dog	Blood	0.790	14.4	3.9	3.7
	Serum (per cc.)	0.199	3.86		
Rabbit	Blood	3.96	15.0	4.5	4.2
	Serum (per cc.)	0.877	3.60		

From: G. B. Wallace and B. B. Brodie, J. Pharmacol. and Exper. Therap. *61*: 407–408. 1937.

Thus iodide, thiocyanate, and chloride were all estimated simultaneously in serum and transudates or cystic fluid. For one guinea pig the paired values for serum: fluid (in milligrams per cc.) were 0.147 : 0.140 for iodide; similarly 0.196 : 0.192 for thiocyanate; and 4.28 : 4.31 for chloride. Homologous values for another guinea pig were for iodide 0.151 : 0.142; for thiocyanate 0.212 : 0.218; and for chloride 3.94 : 4.15. In a human case, the ratio for iodide was 0.077 : 0.073 and for chloride 3.50 : 3.60.

The lymph of normal and of goitrous thyroids has been the subject of a long controversy. At present it is impossible to formulate a final answer to the problem, which is confused by two general types of conflicting evidence. As an example of the first may be cited the classical finding of Hektoen, Carlson, and Schulhof [235] that lymph collected from the main vessel leading from the thyroid of goitrous dogs consistently showed a positive precipitin test for thyroglobulin. Unfortunately, these results have not been confirmed invariably, and the test is not specific for thyroid hormone. An example of the second type is the work of Williamson, Pearse, and Cunnington,[576] who separated "secretion" (or "hyperplastic") tissue from "colloid" tissue. Although this evidence was accumulated for the purpose of proving a dual function of thyroid tissue, in the present connection it provides direct analyses of thyroid parenchyma during a period of marked cellular activity. The great majority of 200 samples of "secreting" tissue showed no iodine and no biological activity (by tadpole test). Taken altogether, the preponderance of negative evidence on the presence of thyroid secretion in thyroid lymph forces one to the verdict of "not proved."

Iodine in Milk

In the normal lactating animal, considerable quantities of iodine are secreted by the breast.[106] In the first day of human puerperium the colostrum yields only 2 to 5 gamma iodine in 24 hours,[373] but the iodine concentration ranges from 8 to 45 gamma per cent.[139, p. 105] When lactation is established, from 20 to 47 gamma iodine may appear in 24 hours in a concentration of 8 to 12 gamma per cent.[310] Thus the monthly excretion of iodine amounts to from 2.5 to 3.5 mg.[552] The chemical status of this iodine is not clear. The major part of milk iodine is attached to organic material, either by chemical combination or possibly by adsorption.[168] Studies of lactating cattle have shown that iodine in the milk varies with the season and with the type of fodder. Seacoast pasturage increases the milk iodine.[478] There is much less iodine in butter fat than in skimmed milk.[378] The nursing infant does not receive thyroid hormone by way of the milk,[124] nor does the milk yield a thyroxine-like fraction after hydrolysis.[151] Iodide feeding increases iodine in the whey.[453]

Iodine in Sweat and Saliva

Iodine is excreted by the skin in variable amounts; data are not available on variations in concentration. In profuse sweating the total in sweat may be over 30 per cent of the total iodine excreted.[168] On the other hand, Szász [534] found practically no cutaneous excretion in the mountains at 1000 meters' elevation.

The concentration of iodine in saliva may vary from 0 to 362 gamma per cent,[499] but the total amounts excreted by this channel are small. After the therapeutic administration of iodine, high concentrations appear in the saliva within a few minutes.

Blood Iodine

Physiological Range. It has been clear, at least since the work of Kendall and Richardson,[278] that normal blood contains iodine in a characteristic concentration.* Normally, this amount is rarely less than 5 gamma per cent or more than 20 gamma per cent, as recorded by the majority of methods now available.[139, p. 91] The blood of various animals is similar to that of man. Of course, the concentration is subject to fluctuation.[380] The level is increased somewhat by an iodine-rich diet; indeed, therapeutic doses of iodides (e.g., 1 gram of sodium iodide) may raise the level temporarily to several hundred gamma per cent. Likewise, a diet of iodine-rich foods, especially marine fish and seaweeds, increases the blood level. Sturm and Buchholz,[529] among others, have found that seasonal variations in blood iodine occur parallel with changes in thyroid iodine. Löhr [342] failed to confirm this, but it should be remembered that in certain localities (e.g., the British Isles) thyroid iodine may not be subject to remarkable fluctuation.[365] The blood iodine may rise at the time of the climacteric in women,[69] but this phenomenon is not constant.

---

* As pointed out in the text, no great importance is attached to the absolute magnitude of figures given for blood iodine concentration except in a few cases where the absolute truth is ostentatiously discussed. In most physiological problems, only relative values, contrasted with a normal control, are needed. In evaluating statements involving iodine concentration, therefore, the reader must become accustomed to thinking in terms of a sliding scale. In nearly every case where iodine concentration is mentioned, the normal control figure is given simultaneously. At the present stage of technical facilities for determining iodine in minute amounts it is necessary to think in terms of relative truth without insisting upon absolute truth.

There is considerable diversity of opinion as to the distribution of iodine between red cells and plasma. It seems clear that the plasma contains the major portion. Representative figures indicate about 8 to 12 gamma per cent in the serum and only 4 to 6 gamma per cent in the red cells.[199] Trevorrow [549] studied a series of analyses of whole blood and plasma and found the total iodine to be distributed in proportion to the water content of the plasma and cells. This statement should be considered a first approximation. (Cf. pp. 76, 96.)

Qualitative Nature of Blood Iodine Compounds. Ironically enough, despite many quantitative studies of the partition of the blood iodine, the nature of these fractions is largely unknown. Various methods have been used to fractionate the blood, but they are so arbitrary that it is difficult to compare results obtained by one method with those by another procedure. A systematic study of the blood iodine by rigorous physicochemical methods is sorely needed. Meanwhile, it is possible to draw tentative conclusions only.

Even if the plasma and red cells contain iodine in equivalent concentration in terms of water,[549] there is no evidence that these concentrations necessarily represent the same chemical compounds. Indeed, Zawadowsky and Asimoff [584] found that, on feeding thyroid, the plasma became biologically active, but *not* the red cells. It will be important to learn whether red cells contain hormone because, if not, the plasma should be analyzed separately, in view of its greater physiological significance.

There are many "normal fasting" values for "inorganic" iodide in the literature, but it is dubious whether much of the normal blood iodine is truly inorganic unless iodide has been ingested recently.[549] Ultrafiltrates of plasma from fasting animals contain little iodine unless potassium iodide has been fed to the animal recently. On the other hand, iodide added to plasma can be recovered quantitatively in the ultrafiltrate. When animals are fed iodides, however, the extra inorganic iodide is distributed between plasma and cells in (roughly) equivalent concentration, i.e., in terms of the respective water contents of the two phases.[374] Although these filtration experiments indicate that most of the normal blood iodine is bound by protein, Trevorrow [549] has found that it can be removed from the protein almost completely by extraction with butyl alcohol or by continuous extraction with ethyl alcohol or acetone. In other words, the union with protein may be due to adsorption rather than to firm chemical combination. Thus by varying the procedure, the normal fasting blood iodine can either (*a*) be left almost entirely adherent to protein, or (*b*) be removed almost completely from protein. Unfortunately, many of the procedures employed in the past 15 years represent intermediate stages. Thus the classical procedure of Lunde, Closs, and Wülfert [347] for the determination of "organische" iodine gave the fraction which happened to be bound to protein after *one* extraction with alcohol. Similarly, McClendon [377] recently has used acetone to find the "hormone iodine" left bound to protein, despite the finding by Eufinger and Schulte [161] that all of the iodine can be removed by acetone if further extraction be made. In other words, it is hazardous to assume that alcohol-soluble (or acetone-soluble) iodine represents "inorganic" iodide, whereas the alcohol-insoluble (or acetone-insoluble) represents "organic" or "hormone" iodine alone. Similarly, the use of dialysis or electrodialysis [45] gives values for diffusible "iodide" which depend upon changing concentrations and rates to diffusion.[160]

Recent analytical studies [549, 428] indicate that thyroxine, diiodotyrosine, thyroxine-proteose and thyroglobulin are all nearly completely precipitated with the proteins of blood by such agents as heat and acetic acid or zinc sulfate and sodium hydroxide. On the other hand, added iodide escapes precipitation. Such observations suggest that the normal fasting blood iodide concentration is only about 1 or 2 gamma per cent, and even in hyperthyroidism this inorganic fraction seems not to differ greatly from the normal unless iodide has been employed. Contrast this figure with the high values (cf. Table 21) for alcohol-soluble "iodide" now in the literature! The author suggests that non-precipitable iodine be regarded henceforth as an index of inorganic iodide.

If this procedure is followed, it seems likely that the statement already made in Chapter II — namely, that the concentrations of iodide in the organism "may possibly be increased several times in hyperthyroidism" — will become superfluous, because it is based upon an analytical method which may ultimately be outmoded.

The protein-bound iodine of the plasma also may prove to be a useful measurement provided care is taken to obtain the maximal value possible. This figure would include the various constituents of thyroglobulin mentioned above, if present, and possibly other unknown iodine-containing substances which can be adsorbed on protein molecules. Indeed, when thyroxine is added to blood it may appear either in the "anorganische" or the "organische" fraction, depending upon the procedure employed.[428] This fact may explain why Leipert[311] was able to fractionate diffusible iodine into fractions precipitable by silver and those not so precipitable. Lipoid-bound iodine also has been suggested,[560] but it seems insignificant if present at all.[311]

TABLE 21

IODINE FRACTIONS IN BLOOD

Normal blood	Average total blood iodine	Average "organische" blood iodine	Average "anorganische" blood iodine
	micrograms	micrograms	micrograms
10 cc. of blood	0.85	...	...
10 cc. of blood + 3.8 micrograms of organic iodine *	4.62	...	...
10 cc. of blood	...	0.82	0.12
10 cc. of blood + 3.8 micrograms of organic iodine *	...	4.46	0.24
10 cc. of blood + 2.5 micrograms of inorganic iodine †	...	0.84	2.65
Blood from clinical cases	**micrograms per cent**	**micrograms per cent**	**micrograms per cent**
Nontoxic goiter (no iodine)	8.0	5.3	3.2
Exophthalmic goiter (no iodine)	16.3	10.0	6.5
Exophthalmic goiter (with iodine)	19.4	8.1	10.8
Nontoxic goiter (with iodine)	17.1	8.7	8.2
Primary myxedema	5.7	2.5	3.1

* Thyroid proteose was used.
† Potassium iodide was used.
From: H. J. Perkin and L. M. Hurxthal, J. Clin. Investigation, *18*: 734. 1939.

Trevorrow[519] has been able to separate the butyl alcohol extract of blood iodine into thyroxine-like fractions and diiodotyrosine-like fractions. She concludes that "the greater part of the blood iodine possesses properties similar to those of thyroxine and diiodotyrosine, and a portion of this is not diiodotyrosine but is thyroxine-like in its solubility."

In this connection, immunological observations recently made by Lerman are illuminating. He produced rabbit antiserum "which would detect human thyroglobulin in concentrations as low as 0.15 to 0.3 mg. per cent, i.e., in a concentration equivalent to less than 1 microgram per cent of iodine. *This was true whether the thyroid protein was dissolved in physiological salt solution or in normal human serum.* He was unable to detect such a concentration in normal human serum, thyrotoxic serum (pre- or postoperatively), or in myxedema. He was unable to detect any thyroglobulin in human urine. One preparation of the antiserum, which was unusually potent, was capable of disclosing a concentration of thyroglobulin as low as 0.05 mg. per cent. With this potent antiserum Lerman was unable to detect any thyroglobulin in serum from hyperthyroid patients." [316] * These observations suggest strongly that the circulating hormone differs from the storage form previously described. It is curious that rabbit's serum containing antibodies against human thyroglobulin does not react with iodinated serum protein, either horse or human. Neither does it react with iodinated horse albumin. It will be remembered that these preparations all relieve myxedema completely. On the other hand, the immune serum does react well with colloid extracted from human colloid goiter, which failed to show any endocrine potency. In short, the immunological specificity has not been found to be related to endocrine specificity. (Cf. p. 99.)

For the sake of uniformity in the literature, the author suggests that in clinical and metabolic studies the following program be followed. (*1*) Plasma to be separated from red cells, at least until it appears that the iodine of red cells reflects precisely that of the plasma. (*2*) The plasma iodine to be separated into "I" iodine, presumably iodide because ultrafilterable or non-precipitable; and into "P" iodine, the maximum adsorbable on protein or precipitable with protein molecules. (*3*) The "P" iodine to be separated, according to Leland and Foster,[315] into a "T," thyroxine-like fraction and "D," diiodotyrosine-like fraction.[425] This plan will admit of direct comparison of concentrations in the blood with those in the thyroid gland itself, or with pharmaceutical preparations and derivatives of thyroglobulin or of iodoprotein.[318, 321] Indeed, in one such experiment with beef plasma, Trevorrow found 92 per cent of the total iodine in the "P" fraction, of which one-third was "T" iodine and two-thirds "D" iodine. These are the values one would have expected from the normal human thyroid in Boston!

---

* It was possible, however, to detect circulating thyroglobulin in the thyroid veins near the end of routine subtotal thyroidectomy, presumably as a result of mechanical manipulation. Occasional traces could likewise be detected in the systemic circulation at this critical time, but this finding was observed to be only transient.

The use of plasma, rather than whole blood, is recommended because it probably gives a wider spread in values and is therefore more sensitive. The following typical values obtained by Miss Sophia M. Simmons in the author's laboratory illustrate this point. Values for "P" iodine in gamma per cent are given for whole blood (b), for plasma (p) and for cells (c), respectively. In myxedema b, 3; p, 2; c, 3. In a euthyroid individual b, 6; p, 6; c, 6. In mild hyperthyroidism (basal metabolic rate equal to plus 30 per cent) b, 8; p, 11; c, 6. In severe hyperthyroidism b, 9; p, 12; c, 6. It will be noted that in mild hyperthyroidism the plasma value may be abnormally high when the value for whole blood is essentially normal. This phenomenon doubtless accounts for some of the "normal" values reported in the literature for cases of obvious clinical hyperthyroidism.

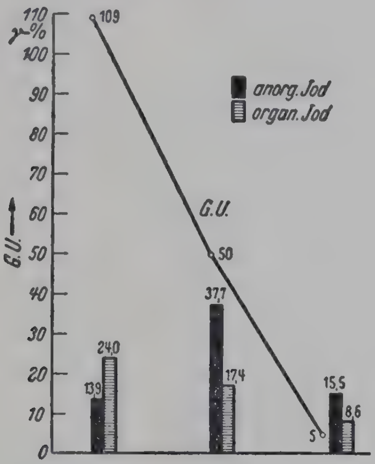
After human blood has been hydrolyzed directly in alkali, it yields both "T" and "D" fractions.[149] Although the hydrolysis admittedly destroys some 20 per cent of added thyroxine, nevertheless it increases qualitatively the reliability of the results. The "T" iodine is between 2.9 and 4.8 gamma per cent, i.e., about one-third of the average total iodine (7 to 12 gamma per cent).* Thus 5 liters of human blood would contain nearly 0.5 mg. of apparent thyroxine; 25 liters of horse blood, 1 mg.; and the entire blood of a dog from 0.05 to 0.025 mg. of apparent thyroxine.

Extant Clinical Data

These academic considerations notwithstanding, there exists a large mass of clinical and metabolic material based on the concept of "anorganische" and "organische" iodine as separated by the alcohol precipitation of Lunde and Closs[346] or by similar procedures. Such values are illustrated in Fig. 7. Because these data convey important information, we must review them carefully, remembering that "anorganische" iodine often contains little iodide and that "organische" iodine represents only a portion of the maximal iodine which is adsorbable on protein. The "organische" iodine is sometimes called "hormone iodine." [377] The consistent behavior of this value in normal and in hyperthyroid individuals offers some hope that eventually this arbitrary fraction may be closely identified with the "T" fraction described above, even though it probably constitutes the net effect of several compensatory errors.

---

* It will be noted that these figures, taken from the general literature, are probably too high, although they serve best as a convenient reference scale for a great mass of data now in the literature. If the reader is interested in absolute truth, these values should be scaled down, as suggested in the footnote at the beginning of this section (p. 71).



Ges.-Jod	37,9 γ-%	55,1 γ-%	24,1γ-%
Therapie	3mal täglich 3 Tr. Lugol, ansteigend auf 3mal tgl. 10 Tr. Lugol		Nach Schilddrüsenresektion
Klinischer Befund	Schwerste Basedowerscheinungen	Erhebliche Besserung	Völliges Wohlbefinden
Zeit	Vor Jodbehandlung	Am Ende der Jodbehandlung (14 Tage)	3 Woch. nach Schilddrüsenresektion

FIG. 7. When a patient with exophthalmic goiter is successfully treated with iodine and the basal metabolic rate falls, the "organische" iodine fraction in the blood frequently falls too, despite a rise in the "anorganische" fraction.

In the figure, G.U. indicates basal metabolic rate. The ordinate scale serves two purposes: it indicates both the basal metabolic rate (in percentage above normal) and the blood iodine concentration in gamma per cent.

From: K. Gutzeit and G. W. Parade, Ztschr. f. klin. Med. *133*: 520. 1937–38.

Fasting Blood Iodine. Because of the technical difficulties encountered in separating small amounts of iodine, not to mention the diversity of analytical techniques in vogue at present, comparative values obtained by the same investigators are more reliable than

TABLE 22

RANGE OF PLASMA IODINE IN BOSTON

Status of patient	Age	Sex	Diagnosis	B.M.R. (%)	IODINE (IN MICROGRAMS PER 100 CC. PLASMA)	
					Total	"P"
Euthyroid *	25	F	Normal	..	9.8	..
"	17	F	Simple goiter. Possible incipient hypothyroidism	−17	8.9	..
"	54	F	Anxiety state	+34	6.8	..
"	25	F	Normal	..	..	5.6
"	37	M	Pneumonococcus pneumonia Type IV	+31	..	5.0
"	69	M	Gen. arteriosclerosis; arteriosclerotic ht. dis.; hypertension; senility; alcoholism	0	..	7.4
"	44	F	Arteriosclerotic and hypertensive ht. dis.; auricular fibrillation and congestive failure	+39	..	9.5
"	52	M	Hypertensive and arteriosclerotic ht. dis.; auricular fibrillation; congestive failure	+27	..	6.2
Graves' disease	17	F	Recurrent toxic goiter; Graves' disease without hyperthyroidism	+9	..	9.3
Hyperthyroid	62	F	Toxic goiter	+36	18.1	..
"	39	M	Diffuse toxic goiter; coronary ht. dis.; old myocardial infarction	+28	..	16.4
"	16	F	Diffuse toxic goiter	+50	..	14.8
"	33	F	Recurrent hyperthyroidism	+60	..	12.7
"	25	F	Diffuse toxic goiter	+66	..	12.1
"	51	M	Diffuse toxic goiter	+80	..	16.9
Thyroid storm	20	F	Thyrotoxic crisis	None	..	18.2
Hypothyroid	28	F	Myxedema	−43	..	3.9
"	16	F	Hypopituitarism; secondary myxedema (mild)	−27	..	4.3
"	41	M	Rheumatic heart disease; mitral stenosis; hypothyroidism (total thyroidectomy)	−30	..	3.4

* Obviously, the "abnormal" values for basal metabolic rate obtained in this group were false, or at least misleading.

In the same laboratory, when the preceding method was further modified by incorporating the titration procedure described by Riggs and Man in the Appendix, the normal range became 4.5 to 8.0 gamma per cent. The latter range probably is nearer the absolute truth, therefore, than the data given in Table 22. In fact, as techniques improve the normal range is becoming lower and more sharply restricted.

absolute values obtained by different procedures in various localities. Obviously, when stating blood iodine in studies of pathological physiology, each investigator should make clear his range for normal values.

As regards whole blood iodine, fasting values obtained by Gutzeit and Parade [213, 212] in Breslau are instructive:

For normal men	9 to 16 gamma per cent (average 14 gamma per cent)
For normal women	11 to 20 gamma per cent (average 17 gamma per cent)
"Organische" iodine	3 to 5 gamma per cent

Normal "iodine quotient" = $\frac{\text{“organische”}}{\text{“anorganische”}}$, 0.2 to 0.5

In Boston, Perkin, Lahey, and Cattell [432] found in normal adults an average of 6.6 gamma per cent, ranging from 1 to 10 gamma per cent. In the author's laboratory, a few miles away, values for plasma "P" iodine in normal individuals are rarely below 6 gamma per cent, or above 10, by the method of Trevorrow and Fashena,[550] slightly modified. Characteristic values are shown in Table 22. In Ohio, Davis, Curtis, and Cole [121] found in normal adults an average of 10.1 gamma per cent, ranging from 8.5 to 16.2 gamma per cent. In children in New York City, Fashena [165] found average values, without sex difference: for infants less than 24 hours old 4.7 ± 0.33 gamma per cent, ranging from 1.0 to 11.0 gamma per cent; for others up to 13 years, 6.6 ± 0.15 gamma per cent, ranging from 3.0 to 12.0 gamma per cent. Similarly, from the New York Postgraduate Hospital data were reported at the New York Academy of Medicine's Twelfth Graduate Fortnight in 1939 as follows: Simultaneous iodine contents of blood and urine were determined in 22 normal individuals and values were given for (*a*) blood iodine concentration, (*b*) urinary iodine concentration, and (*c*) total 24-hour urinary iodine excretion. The range was, for (*a*) 3.1 to 8.4 gamma per cent; for (*b*) 6.2 to 56.7 gamma per cent; and for (*c*) 18 to 483 micrograms. The corresponding means were for (*a*) 5.9 ± 1.3 gamma per cent; for (*b*) 21.1 ± 16.7; and for (*c*) 190.8 ± 138 micrograms (M. Bruger).

For comparison, values for "organische" and "anorganische" blood iodine concentrations in Boston are shown in Fig. 8.

Effect of Exercise. In studying blood iodine, it must be remembered that vigorous muscular activity causes an increase in the fasting blood iodine within a few minutes. There is disagreement about the extent to which the iodine rises; McCullagh and McCullagh[382] report a rise of about 9 gamma per cent, whereas Herxheimer, Mislowitzer, and Stanoyévitch[242] found increases of over 150 gamma per cent. The normal level is not restored for over two hours after cessation of the work.

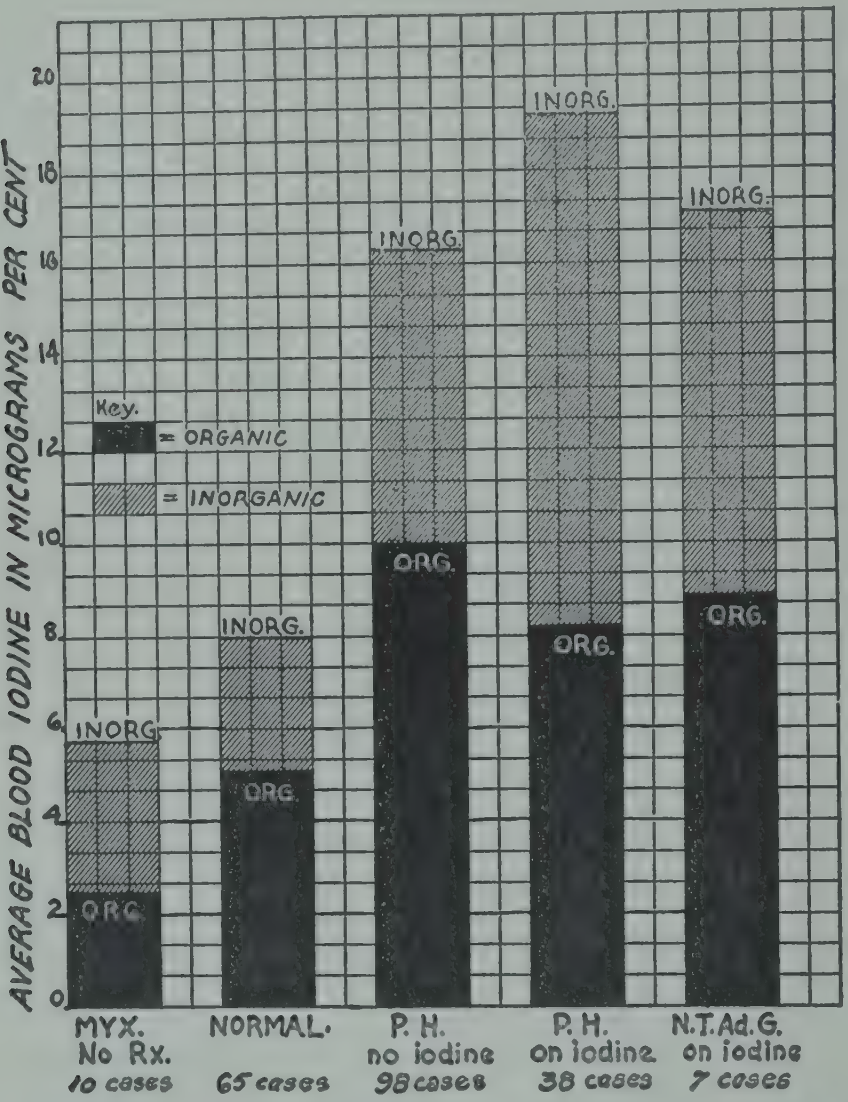


FIG. 8. "Organische" and "anorganische" blood iodine fractions in human individuals. The key to the diagnosis follows: MYX, myxedema; P.H., exophthalmic goiter; N.T.Ad.G., nodular goiter (non-toxic).

From: H. J. Perkin and L. M. Hurxthal, J. Clin. Investigation, *18*: 736. 1939.

Fluctuations in Blood Iodine

Thus far, we have been concerned mainly with blood iodine in the fasting state. Obviously, marked changes in blood iodine may occur when iodine is administered or when unusual amounts of iodine are released from endocrine glands or other tissues.

Circulating Iodine after Administration of Iodine Compounds. The total blood iodine concentration at a given moment represents the net result of many complicated processes. Among these are the quantity and type of compound used, the rate of intestinal peristalsis (i.e., presence of diarrhea), the ease and rate of intestinal absorption or of parenteral injection, the avidity of the thyroid for new iodine, the permeability of various tissue cells, the state of kidney function, and many other factors. Furthermore, different species of animal react differently. Consequently, manifold variations are observed and it is possible to consider only certain typical combinations of circumstances.

Iodide Administration. Potassium iodide is so rapidly absorbed that the blood iodine concentration rises to a peak within 30 minutes if doses of 2 grams or more are ingested.[167] Under these circumstances, the blood iodine concentration may rise well above 100 gamma per cent. With smaller doses (e.g., 0.5 gram) the peak is not reached for 90 minutes.[560] Then the blood iodine falls, to reach the initial level in 48 hours. Thus the bulk of the iodine appears rapidly in the circulation and again disappears rapidly.[135] In a later section, iodine tolerance curves are discussed to show that the functional activity of the thyroid regulates this fluctuation in blood iodine to a large extent. Eventually a major part of the extra iodine is eliminated through the kidney. A much smaller amount is eliminated into the intestine through the bile and intestinal secretions. Possibly the stomach also eliminates important amounts of iodine,[326] thus adding to the intestinal content.

After intravenous injections of iodine, the blood level drops rapidly from its initial peak. The type of curve obtained, however, is determined by the dose injected. Elmer[139, p. 155] has injected small doses (1.7 mg. potassium iodide) and found that in normal individuals the blood iodine returns to control levels in six hours. Perkin and Lahey,[433] using larger doses (i.e., 0.6 gram by mouth), found a more protracted hyperiodemia, as did Fitz.[174]

Even though the total blood iodine rises, the "*organische*" moiety thereof falls,[560] as shown in Fig. 7. Thus, after the oral administration of 0.5 gram of potassium iodide the "organische" moiety may drop from 10 gamma per cent to 3 gamma per cent when the total iodine approaches its peak. Later, the total iodine approaches the normal after 48 hours, as does the "organische" moiety. Thus the curves for total and "organische" iodine, respectively, at first separate and then approach each other again. It may be that the reason for the drop in "organische" iodine is suppression of the thyroid secretion.[527, 346] If true, the rapidity of the fall in concentration is remarkable because thyroid changes are usually considered sluggish.

Administration of Thyroxine Derivatives. The blood iodine curve following the administration of whole thyroid or of thyroglobulin has not been studied adequately. Veil and Sturm,[560] however, studied the effect of feeding small doses of a desiccated preparation called "thyroidin" (containing 150 gamma iodine). In two hours the peak was reached at a questionable 41 gamma per cent, and in 24 hours the blood was normal. More studies are needed on this point. Both "organische" and "anorganische" fractions increase, suggesting that decomposition of the hormone occurs in the gastrointestinal tract, in the liver,[153] or elsewhere. Nevertheless, after the enteral administration of thyroglobulin some antigen (presumably thyroid protein) is found in the blood stream.[236] Most of the ingested thyroglobulin is split, however, as expected, into peptones or peptides in the gastrointestinal tract.[33, 34]

Thyroxine per Os. When thyroxine is administered by mouth appropriately dissolved in alkali,* the blood iodine of dogs reaches 28 gamma per cent in two hours and falls to normal in 12 hours.[489] After injecting 2 mg. of thyroxine, Böe and Elmer[52] found that there was an almost immediate fall from the initial peak of 36 gamma per cent. In 24 hours the blood was again normal. It is highly significant that the rise was entirely in the "*organische*" moiety.

Hypothyroidism

Etiologically, hypothyroidism may be primarily concerned with thyroid disease or the chief cause may lie outside the thyroid, which becomes inactive secondarily. In the second group such causes as iodine lack, tyrosine deficiency and pituitary failure come to mind. In both groups the concentration of circulating hormone in the blood falls.

---

* Thompson and his collaborators[545] have shown that about 80 per cent of the drug is assimilated, when administered in this fashion to human patients.

A study of blood iodine in hypothyreosis is particularly interesting because it should give some idea of the normal hormone content by the difference in total iodine values. In myxedema and in endemic cretinism, values of about 6 gamma per cent are common.[133, 382] The value is so low that it is easily affected by iodine in the environment and by analytical technique. Two representative series for myxedema show values ranging from 4 to 6 gamma per cent and from 1.4 to 11 gamma per cent,[155, 112] respectively. These values presumably reflect non-hormonal iodine, chiefly based on exogenous and endogenous (tissue) metabolism. Gutzeit and Parade[212] found, in general, a low normal "organische" iodine concentration, but found in one case of myxedema only 1.8 gamma per cent.

The plasma "P" iodine, as determined in the Thorndike Memorial Laboratory, has been consistently low, namely 2 to 3 gamma per cent. Presumably this latter value is close to the absolute truth. The "T" fraction probably is almost nil.

Assimilation of Iodine. When iodine-containing food is given to a thyroidectomized dog, it causes a marked rise in blood iodine concentration. Thus two hours after feeding, the blood of a normal dog shows at most 20 gamma per cent iodine, whereas in the athyreotic dog the level reaches about 250 gamma per cent. When pure potassium iodide solution is administered to athyreotic organisms, however, no difference from the normal may be detected *unless* special conditions are imposed. Using small doses of iodide in human myxedema, Elmer[139, p. 446] has found that the blood iodine curve is maintained at higher levels and for a longer time than in normal men. The normal blood reaches its previous, control level in six hours; the blood in myxedema not for 24 hours. This striking inability of the organism to store iodine may be ascribed not only to lack of thyroid tissue, but also to the abnormal state of other tissues, as discussed in Chapter XI (see Fig. 38).

To be sure, other authors, working under different conditions, have found either no such difference from the normal or even a reverse effect. Thus de Quervain and Smith[443] gave potassium iodide daily for a week to cretins and to normal controls. Then the cretinoid blood iodine was 6.4 gamma per cent, duplicating the initial value of 6.4 gamma per cent. By contrast, the healthy blood was 15.4 gamma per cent, as against the initial 13.2 gamma per cent. Likewise, thyroidectomized animals in some series have shown little difference from the normal behavior.[374, 526] The extra iodide may even disappear from the blood more rapidly than in the normal animal.[297] Obviously, the result of these observations depends upon such variables as dosage and time. On the whole, Elmer's results [137] are consistent and, as will be shown, of clinical interest.

When thyroxine is administered to a myxedematous man, the blood iodine increases. Thus 2 mg. of thyroxine, given intravenously daily for several days, elevates the blood iodine from 6 to 17 gamma per cent;[133] thereafter the level becomes stable at 11 gamma per cent. On the contrary, in a healthy individual no change can be noted even after repeated injections for six days. Under such treatment of clinical myxedema, the basal metabolism in the early days overshoots the rise in blood iodine (which is linear with time).[159] Simultaneous determinations of "thyroxine-like" iodine would be valuable.

Effect of Thyrotropic Hormone. Because the thyroid is usually completely atrophic in myxedema,[416] administration of thyrotropic hormone produces no rise in blood iodine.[490] In milder cases of hypothyroidism, in which marked destruction of thyroid follicular cells has not occurred, the thyrotropic hormone may increase the blood iodine and the basal metabolic rate,[139, p. 456] at least temporarily. In unfavorable cases, no rise in basal metabolism occurs, even though the blood iodine may rise somewhat, e.g., from 6 to 16 gamma per cent.[139, p. 457] Consequently, whole thyroid substance or thyroxine usually affords more prompt and less expensive relief in myxedema than does thyrotropic hormone.

Hyperthyroidism

In hyperthyroidism there is usually a definite increase in blood iodine concentration.[560] In the less severe cases it may be difficult to discover because the normal level for the individual may not be known. Likewise, in iodine-poor districts, blood levels in hyperthyroidism may appear rather low because the base line of reference for the region is *very* low.[381] Blood iodine concentrations of 21 to 22 gamma per cent are common in exophthalmic goiter,[560] but the values rarely exceed 100 gamma per cent.[112] * Some authors have found normal values, e.g., 14 to 16 gamma per cent, in 10 per cent of their hyperthyroid cases. Possibly such individuals had an unusually low (normal) blood iodine initially. Of peculiar interest are the "toxic" cases reported by McCullagh [381] and by Perkin, Lahey, and Cattell,[432] in which blood iodine was normal. These were long-standing cases, in which presumably a state of iodine depletion or exhaustion had ensued. Similarly, Zondek [586] has reported low blood iodine in "coma basedovicum." These exceptional cases, showing clinical "thyrotoxicosis" with continued normal blood iodine, suggest that hyperthyroidism may be merely a (major) incident in clinical Graves' disease.[587] Perhaps independent primary neurogenic activity of the hypothalamus sets a minimum level to which "basal" metabolism can be reduced despite block, suppression or extirpation of the thyroid.[173]

Role of the Thyroid in Hyperthyroidism. In general, in clinical hyperthyroidism two features tend to disturb the relation between the clinical condition and the blood protein-bound iodine. One is the lag between increase in blood iodine and the tissue response. The other is the influence of nervous activity, which may induce a clinical state called "toxicity" even when blood iodine is relatively low and the true, or best obtainable basal metabolic rate is also rather low. Falta and Fenz [164] accordingly describe clinical "thyrotoxicosis" as a complicated disease in which, at one extreme, illness exists without much disturbance in thyroid function and, at the other extreme, a high calorigenic effect is found without marked nervous activity. As implied by definition, true hyperthyroidism seldom exists without an elevated blood hormone concentration when measured from an appropriate baseline. This "thyroid diarrhea" of Kocher results in histological hyperplasia as the gland tries to maintain the pace.

Recent experiments by Loeser [338] indicate that a rather permanent hyperthyroidism may be produced by continued injection of increasingly large doses of thyrotropic hormone. This finding has been verified by Elmer,[145] who showed also that continued hyperthyroidism produced characteristic changes in the heart and thyroid of guinea pigs. Interestingly enough, in a recent review Means summarizes the latest evidence of this sort and concludes that in human hyperthyroidism the excessive activity of the gland is due chiefly to stimulation by the tropic hormone of the pituitary.[386]

---

* Such values are probably fantastically high and must be interpreted with a sliding scale in mind, as discussed in the footnote on page 71.

Relation of Blood Iodine to Basal Metabolic Rate. It is generally stated that the blood iodine level does not run closely parallel to the severity of the disease. Nevertheless, Möbius and Nolte[398] found a certain correlation between the increase in basal metabolic rate and the increase in blood iodine concentration. The author suggests that the correlation might be greater if the logarithm of the plasma

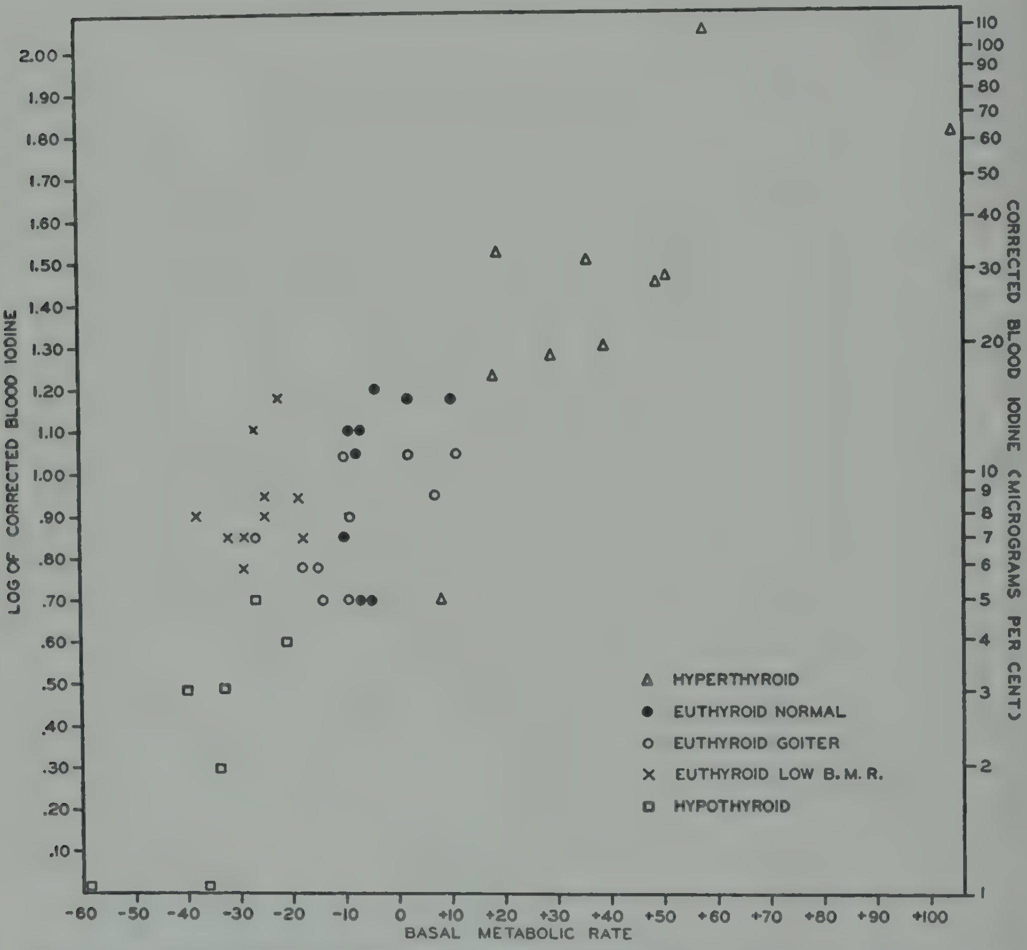


FIG. 9. Blood iodine and basal metabolism. Data for blood iodine concentration in several states of thyroid function, collected by Elmer and Scheps in Lwów. An arbitrary correction of 3 gamma per cent for inorganic iodide has been subtracted from the total blood iodine. The scale showing iodine concentration is logarithmic. The high correlation with basal metabolic rate leads to the inference that the two are related if (*1*) clinical classification is adequate; (*2*) analytical technique good; (*3*) exogenous iodide intake is low; and (*4*) a steady physiological state is attained before analysis is made. (See McClendon, Foster and Kirkland, 1940.)

From: W. T. Salter, Physiol. Rev. *20*: 361. 1940.

concentration be studied. He has compiled in such fashion the data of Elmer and Scheps,[155] who stated that they failed to find a close parallelism. These data show a considerable fidelity to the equation:

$$\text{B. M. R.} = \log\ (\text{Blood Iodine minus } 3)$$

when blood iodine is expressed as micrograms per 100 cc. This relationship is shown in Fig. 9. The coefficient of correlation $r = 0.75 \pm 0.07$ and P is less than 0.01; i.e., the chances that this correlation is significant are better than 100 to 1.

When the "organische" moiety of the blood iodine is investigated, it is generally found to be increased more consistently than the total iodine.[560] Gutzeit and Parade [212] have tabulated the ratio of "organische" to "anorganische" iodine. The normal quotient is 0.2 to 0.5, whereas in severe hyperthyroidism it may approach 4.0. Sometimes, however, the basal metabolic rate is found to be markedly increased with a fairly low "organische" iodine fraction.[45] Likewise, the inverse combination may occur.[123] Various factors conspire to obscure this relationship, viz., inaccuracy of present analytical methods, the possible presence of inactive organic iodine

TABLE 23

The "Iodine Quotient" in Normal and Hypertensive Individuals

No.	Total blood iodine γ %	"Iodine quotient"	No.	Total blood iodine γ %	"Iodine quotient"
			Normal		
	Men			Women	
1	13.0	0.45	1	15.8	0.20
2	13.3	0.18	2	16.0	0.25
3	14.4	0.40	3	17.5	0.19
4	15.7	0.40	4	18.1	0.17
5	16.2	0.23	5	18.4	0.31
6	17.1	0.35	6	19.1	0.25
7	17.7	0.40	7	22.2	0.34
			Hypertensive		
	Men			Women	
1	20.6	0.24	1	12.9	0.43
2	21.2	0.21	2	13.6	0.31
3	24.0	0.25	3	19.3	0.27
4	25.2	0.46	4	19.5	0.30
5	30.6	0.19	5	21.3	0.36

From: K. Gutzeit and G. W. Parade, Ztschr. f. klin. Med. *133:* 523–525. 1937–38.

compounds (possibly degradation products of the hormone) and the difficulty of attaining in these patients a "steady state," physiologically speaking.

As shown in Table 23, the iodine quotient in hypertensive patients is similar to that of euthyroid individuals, despite the opinion of some endocrinologists that hypertension and hyperthyroidism are related disturbances.

Gutzeit and Parade [213, 212] have laid great stress upon the behavior of the "organische" iodine and of their "iodine quotient," i.e., "organische"/"anorganische" fraction, as shown in Table 24. In severe hyperthyroidism, these measurements indicate that the gland is working at maximal capacity, as indicated by the response to exercise. In normal persons, within an hour or two after vigorous exercise the "organische" iodine rises, and likewise the iodine quotient, to return to normal after 24 hours. In hyperthyroidism, on the contrary, there is no further rise and there may even be a fall in these values.

TABLE 24

BASAL METABOLISM AND "IODINE QUOTIENT"

No. of cases	Elevation of basal metabolism	"Iodine quotient"	
	Up to	Average	Range
11	+ 40%	0.9	0.4–2.3
22	+ 60%	1.25	0.4–2.0
28	+ 80%	1.1	0.6–2.7
7	+100%	1.7	0.8–2.6
2	+110%	1.6	1.5–1.7

From: K. Gutzeit and G. W. Parade, Ztschr. f. klin. Med. *133*: 524. 1937–38.

Elmer, Rychlik, and Scheps [152] attempted to simplify the problem by determining the level of "thyroxine-like" iodine substances in the blood after hydrolysis. They used the method of Leland and Foster, slightly modified. In three cases studied, the blood contained from 9 to 16 gamma per cent of "thyroxine" iodine, i.e., 50 to 60 per cent of the "organische" iodine, or 27 to 44 per cent of the total iodine. Apparently a thyrotoxic man has from 0.8 to 1.4 mg. of thyroxine in his blood, as compared with 0.4 to 0.5 mg. in the normal person. More recently, Trevorrow [549] has improved the analytical technique so that, as data continue to accumulate, it may be possible to demonstrate a closer parallelism between the basal metabolic rate and the concentration of presumable hormone or some mathematical function of it.

Thyroidal Iodine. Ordinarily, the iodine content of blood in thyroidal veins is greater than that in the general circulation or in the thyroid arteries. Breitner[64] described a "flushing out" of iodine as blood flowed through the hyperactive gland, not equaled in euthyroidism. His finding of periodic releases of iodine raises the question, advanced also by Sturm, that the secretion of the thyroid gland may undergo periodic fluctuations, occurring over rather short intervals of time.

For many years the theory of "dysthyroidism" was in vogue, signifying that in thyrotoxicosis an abnormal, highly poisonous, hormonal substance was being liberated from the gland.[441, 397] It has not yet been possible to test the blood directly. Studies of the thyroid itself, however, have failed to reveal an unusually toxic substance. If the hyperactive gland be removed before iodine therapy and tested for pharmacological potency, it yields only a poor calorigenic response and no toxic effect.[301] After 10 days of iodine therapy, Eppinger and Salter[159] isolated the thyroglobulin from human glands and obtained a classical response in human myxedema without untoward effects. Thus there is no tenable evidence of "dysthyroidism" in exophthalmic goiter.

Iodine Therapy. The classical clinical response of Graves' disease to iodine therapy is accompanied by changes in the various fractions of the blood iodine. Of course, the inorganic iodide rises rapidly, but the most interesting point is that the "organische" fraction decreases at once.[251, 252] As yet, the falling "organische" moiety has not been fractionated into "thyroxine-like" iodine and other iodine.

Despite the fact, mentioned earlier, that the "organische" and "anorganische" blood fractions are highly arbitrary, the results of Gutzeit and Parade[213, 212] suggest strongly that these values do indeed reflect physiological changes in the organism. After the injection of inorganic iodine in 13 cases of frank hyperthyroidism, the high iodine quotient fell markedly in all cases, and in 12 cases the "organische" fraction dropped. Of course all patients showed an increase in the total blood iodine which ranged from 9 to 52 gamma per cent. Clinically, it was noted that when the "organische" level decreased, the basal metabolic rate and the pulse rates did likewise, as shown in Fig. 7. Metabolism might decline, however, without a drop in "organische" iodine.

It is essential to distinguish carefully between acute, short courses of iodine therapy and the effects of long-continued iodine administration. This contrast can be observed when diiodotyrosine is given in a dose of 0.1 gram daily.[212] The *acute* effect is usually a proportionate fall in "organische," "anorganische" and consequently in total iodine concentrations. *Chronic* administration (i.e., from 5 to 12 weeks) leads to a rise in the "organische" and total iodine concentrations, and often in the "anorganische" fraction. In short, the *acute* effect is reversed and the iodine quotient rises. Simultaneously, clinical exacerbation of the disease sometimes occurs.

Surgical and Radiation Therapy. Successful surgical treatment likewise reduces the blood iodine.[548] After ligation of both superior thyroid arteries, both the total iodine and the "organische" iodine fall gradually over the course of two weeks, i.e., from 74 to 45 gamma per cent (total iodine). After subtotal thyroidectomy, within a few days the blood iodine falls parallel with clinical improvement.[213] Both the "organische" and "anorganische" fractions share in the decline.

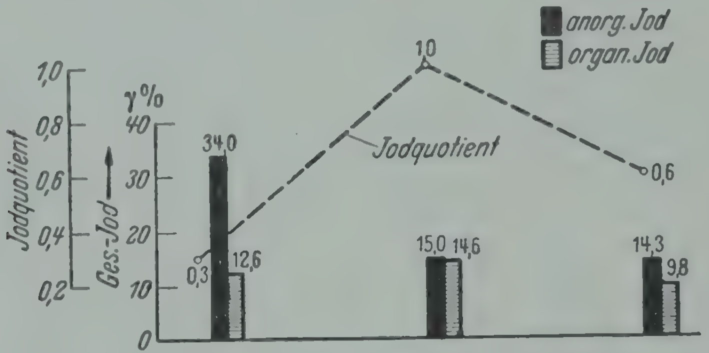
Gutzeit and Parade [213] point out that the indiscriminate use of iodine medication greatly disturbs endogenous iodine metabolism and the iodine pattern in the blood. Thereby queer discrepancies are produced between the chemical findings and the clinical status of hyperthyroid individuals. If one is careful, however, to compare the chemical findings in florid Graves' disease before iodide therapy with similar findings observed several weeks or months after the intervening total thyroidectomy, the results are decisive and consistent. By the time the basal metabolic rate has reached normal limits the "organische" iodine has become normal and the iodine quotient is within normal limits. The "anorganische" fraction, however, may be only slightly less, so that the total iodine gives little evidence of marked improvement in the patient.

In cases successfully treated with iodine before operation, the "organische" blood iodine falls or remains stationary so that the balance between sympathetic activity and endocrine activity is at a nearly normal status by the time of operation. In other words, under iodine therapy the hyperrrheic thyroid has been transformed into a hyporrheic storage gland. On the other hand, if the blood "organische" fraction remains high and increases before operation, the operative hazard is bad because the organism continues to be thyrotoxic. Thus appropriate chemical studies before operation may help to assess the operative hazard. As described in Chapter XI, continued chemical study before and after operation may assist in formulating an opinion as to later progress.

In successfully prepared cases, the low "organische" iodine often continues after operation. Thus the operative procedure merely establishes *anatomical* exclusion of the thyroid in place of the previously existing *chemical* exclusion of the gland from bodily economy. Because even arterial ligation may precipitate a thyroid crisis, it is conceivable that not much increased danger is incurred by removing the gland subtotally when "organische" iodine is low.

After treatment of the gland in hyperthyroidism with x-ray or radium, the blood iodine falls rapidly to about 8 or 9 gamma per cent,[45] from a median value of nearly 26 gamma per cent in the whole blood. On more careful study during the initial x-ray reaction in the tissue, however, an initial increase both in total and in "organische" iodine may occur within two hours unless these levels were very high previously.[213] Perhaps this phenomenon partly explains the rare thyroid storm after x-ray therapy. This acute rise is only temporary, however, and soon a marked fall in both levels follows as clinical improvement ensues. Of course, if acute clinical exacerbation of the disease occurs later, a coincident rise in the blood level ensues.[491]

Thyroid "Storm." The cause of the dangerous postoperative "storm," or "shock" (so-called) has been the subject of many speculations and conflicting data. Recent data, however, suggest strongly that a sudden flooding of the circulation with hormone may occur at operation and so induce the dreaded "storm." Even uneventful cases may show a transient postoperative elevation in blood iodine,[114] illustrated in Fig. 10. The blood iodine level rises in the first few hours after operation (e.g., from 22 to 40 gamma per cent) and then drops.[27] Concomitant depression of the blood cholesterol and elevation of the blood sugar accompany the increased level. In four of eight cases studied by Gutzeit and Parade [213] the "organische" iodine rose within two hours after operation, and these four cases showed increased tendency to postoperative disturbances, perhaps due to a mechanical "squeezing out" of hormone from the gland. Apparently the blood iodine may subside before the storm breaks in the tissues, that is, blood iodine may fall before symptoms become marked. It is interesting that postoperative "shock" after operations upon the thyroid may be relieved by the intravenous administration of iodine.[581] Presumably, this beneficial effect is due merely to the prevention of further increase in circulating hormone.



Ges. Jod	46,6 γ-%	29,6 γ-%	24,1 γ-%
Zeit	Vor Operation	2 Tage nach Schilddrüsenresektion	3 Wochen nach Operation
Therapie	Am Ende der Lugolbehandlung vor Operation	Lugol abgesetzt	Lugol abgesetzt
Klinischer Befund	Erhebliche Besserung des Allgemeinbefindens	Patient fühlt sich postoperativ nicht wohl	Wohlbefinden

FIG. 10. When a patient with exophthalmic goiter is subjected to thyroidectomy, the surgical manipulation may lead to a transitory increase in the "organische" blood iodine and the "iodine quotient." The ordinate shows total iodine.

From: K. Gutzeit and G. W. Parade, Ztschr. f. klin. Med. *133*: 528. 1937–38.

Accordingly, iodide therapy in thyroid storm must be considered merely as a prophylactic measure against further, later increase in the degree of hyperthyroidism.

Drugs and Hormones. The blood iodine in hyperthyroidism may be influenced very slightly by drugs. Likewise, the abnormal metabolic rate can be altered by hypothalamic anesthesia. Thus large doses of "veronal" or "prominal" (0.6 gram daily) definitely depress the basal metabolic rate and may depress the blood iodine concentration slightly. Curiously enough, the cholesterol and carotene may become depressed as the patient improves clinically.[173] At best, however, drugs appear to be of minor importance from the standpoint of practical therapy.

Apparently, prominal does not depress thyroid function per se, but does depress the irritability of centers in the hypothalamus.[213] If these centers exert a constant stimulating effect on the thyroid,[173] the net effect of lengthy anesthesia does not disclose this, because the total iodine falls only slightly. Neither the "organische" fraction nor the quotient changes appreciably. The metabolism *does* tend to fall, but the result can be explained on a nervous basis exclusive of thyroid activity. Similarly, Veil and Sturm [560] found that after the administration of 7 grams of digitalis the blood iodine decreased from 40 to 15 gamma per cent.

Intravenous adrenine *in hyperthyroidism* causes a sudden drop in blood iodine, e.g., from 25 gamma per cent to 5 gamma per cent.[492] After 24 hours the blood iodine level is normal again. On a preceding page it has been emphasized that this effect is opposed to that in hypothyroid cases. The effect of adrenine simulates that of vigorous exercises, and discloses the same discrepancy between the normal and the hyperthyroid gland.[212] Indeed, adrenine gives an indication of thyroid functional reserve in the following manner: the subcutaneous injection of 0.5 mg. in normal individuals produces a rise in "organische" iodine coinciding with the peak of the circulatory response (after 10 to 20 minutes), although the total iodine of the blood does not change much. In hyperthyroid individuals, on the contrary, the thyroid presumably is incapable of further activity, because no extra rise in "organische" iodine occurs, despite a clear rise in blood pressure.

Little is known about the effect of other hormones upon the increased blood iodine, although there may be a depressant effect of ovarian hormone (i.e., estrin) in clinical hyperthyroidism.[517] Likewise, Cucco [110] found that the hyperiodemia of the menopause was diminished by treatment with ovarian extracts.

In recent years several antithyroid substances have been described. Cameron [71] reported that in hyperthyroidism both the increased blood iodine level and the basal metabolic rate were diminished by the use of such material. Likewise, Blum [47] and Collip and Anderson [97] have used antithyroid substance and antithyrotropic serum, respectively. The rationale for using the latter would seem to apply only in disturbances like acromegaly or perhaps like simple goiter, which involve pituitary hyperactivity. Indeed, thyrotropic hormone is believed to be deficient in Graves' disease by some investigators.[239] Anselmino and Hoffmann [17] have studied Blum's antithyroid substance. It occurs in normal blood serum and in the bone marrow. The material is obtained as an ether-acetone extract of normal serum. Its concentration in the blood is reduced in patients with hyperthyroidism. Its mode of action is not understood, but apparently it acts more strongly against the thyrotropic hormone than against the thyroid hormone.[171] In all the controversial evidence bearing on antihormones, there is no clear demonstration of immunity to a specific, iodine-containing prosthetic group.[572, 99]

Simple Goiter

In simple goiter with accompanying euthyroidism, the blood shows generally a low normal concentration of iodine.[113] Such glands merge imperceptibly into the group in which there is hypofunction (i.e., in nodular goiters of long duration), with a consequent tendency toward a potentially hypothyroid state of the organism. In cases with normal basal metabolic rates, Elmer and Scheps [155, 139, p. 514] found the blood iodine to be from 8 to 14 gamma per cent; in cases with subnormal basal metabolisms the values were 8 or 10 gamma per cent. Obviously, there is no clear difference. Furthermore, the plasma "P" iodine remains within normal limits for a long time.

Problems Concerning the Iodine of the Blood

In a recent review on the level of iodine in the blood, Perkin and Lahey [433] have pointed out the present "difficulty in determining the absolute amount of iodine in blood. However, the fact is not precluded that the results of determinations of iodine in the blood of different patients or of the same patient at different times are not relative one to another when estimated by the same method and by a worker familiar with the method. The relative blood iodine values have added considerably to the present conception of iodine metabolism in goitrous conditions." As improved, iodine-free reagents have became available, and as technical advances in the micro-estimation of iodine have been made, there has been a gradual decrease in the apparent concentration of normal blood iodine. In consequence, many of the "normal" values cited in the preceding pages would indicate markedly elevated concentrations, if judged by the latest standards. Two recent series may be cited as examples.

Very recently Turner, DeLamater, and Province[551] have employed the "closed" method of Trevorrow and Fashena (see Appendix) and have reported a series of determinations on normal individuals living in New York City. The blood iodine of twenty such normal males ranged from 3.8 to 8.6 gamma per cent, with an average of 5.9. Similar values for normal females were 3.5 to 10.4 gamma per cent, averaging 6.8. No seasonal variation could be demonstrated. These authors arbitrarily set their "normal range" at 4.0 to 10.0 gamma per cent. It should be noted that these values are for total iodine and do not refer to the organic fraction alone.

Likewise, Perkin and Lahey[433] reported analyses of total blood iodine by the open-ashing method (see Appendix)

> made on 1,823 persons. In 745 persons who presented no clinical evidence of thyrotoxicosis the average (or mean) level of iodine in the blood was 6.8 micrograms per hundred grams. The values for blood iodine ranged from 2 to 15 micrograms per hundred grams of blood, with 61 persons (8.2 per cent) having a value in excess of the upper normal limit by the method (10 micrograms per hundred grams). The results showed no significant variations attributable to age, sex or season. It was found generally that normal persons of a vagotonic type had blood iodine values within the lower normal range. On the other hand, normal persons of a sympathicotonic type were found usually to have blood iodine values within the upper normal range. Since the same person varies in temperament, the level of iodine in the blood may fluctuate in a similar manner. The correlation, however, could not be considered absolute.

The blood iodine values for 1,078 patients with clinical hyperthyroidism were also known.

> The average value for blood iodine was 15.5 micrograms per hundred grams. The mean level was considerably less, 11 micrograms per hundred grams. The range was from 2 to 100 micrograms per hundred grams. Approximately 34 per cent of the patients had blood iodine values of less than 10 micrograms per hundred grams. As previously pointed out, the presence in a specific case of an elevated or normal blood iodine value was roughly dependent on the duration of the hyperthyroid syndrome. Although the level of iodine in the blood of patients with thyrotoxicosis may be influenced by states of excitement or of rest, the degree of variation was not as great as in normal persons. The ages of the patients under consideration ranged from 3 to 80 years, with no difference apparent in the effect of hyperthyroidism on the level of iodine in the blood.

The values obtained in these two series assume increased importance when compared with averages of Gley and Bourcet,[198] who used a liter of blood for analysis. In 1900, they reported that dog's blood contained some 55 micrograms of iodine per liter, i.e., 5.5 gamma per cent. Nearly two generations have been spent in reducing the requisite aliquot sample of blood to 10 cc.!

More study of the partition of iodine between cells and plasma is needed, both in a qualitative and a quantitative sense. Most of the analyses now reported in the literature involve the two phases of whole blood, whereas it is not yet clear that the native iodine is uniformly distributed. In fact, recent studies at the Thorndike Memorial Laboratory by Dr. Albert H. Coons and Miss Sophia M. Simmons indicate that the ratio of plasma "P" iodine to cell "P" iodine is as follows: for myxedematous individuals 0.9, for euthyroid individuals 1.0, and for hyperthyroid individuals 2.1. Of course, these ratios apply only to blood sampled in the fasting state, and are not applicable when large excesses of iodide have been administered. Perhaps these ratios will help to explain why in many series values for hyperthyroid patients actually overlap those for euthyroid individuals, even though the upper limit in hyperthyroidism far exceeds normal.

In Chapter XI, more will be said about this group of patients with clinical Graves' disease who show a normal blood iodine. Several possible explanations suggest themselves.[283] In theory, Graves' disease may exist with or without hyperthyroidism.[327] Ordinarily, of course, the thyroid is extremely active functionally and pours out its store of hormone into the circulation. When the thyroid reserve is exhausted, however, and the body's stores of iodine are depleted, may not the primary disease continue, without very excessive utilization of thyroid hormone? Or perhaps, instead, such cases have a high "T" iodine fraction with little or no "D" iodine. Perkin and Lahey refer to such a state as a "relative increase" in organic blood iodine "when the total blood iodine is still within normal limits." Less likely is yet another possibility, namely, that excessive utilization of thyroid hormone may occur without any increased concentration of thyroid hormone in body fluids or even without an excessive excretion of iodine. Obviously, more crucial experiments must be made.

In particular, a better understanding of the qualitative significance of "organische" iodine is sorely needed. At the present time most of the available data are based upon the alcohol separation used by Lunde, Closs, and Pedersen.[346] The author believes that this method gives a false separation of the blood iodine. For this reason the terms "anorganische" and "organische" are used in this volume to indicate results obtained by this European method, in contrast to the terms organic and inorganic, which are used whenever the author believes that the partition of physiological iodine has been made more appropriately.

Of course, much work remains to be done with the "T" and "D" fractions in the plasma of clinical cases. A simple procedure by which they can be separated is outlined in the Appendix, but doubtless it will be improved upon in the next few years. In general it appears that, as compared with the normal proportion between "T" and "D," the "T" fraction is disproportionately exaggerated in frank hyperthyroidism. In myxedema, on the other hand, the "T" fraction approaches the blank of present-day analytical technique. When iodide is administered in excess for several days, the "D" fraction often increases markedly, even in normal individuals and in myxedematous patients. This increase may be minimal or absent in florid Graves' disease; and concomitantly in Graves' disease the "T" fraction characteristically falls under Lugol's therapy.

Recent work by Dr. Albert H. Coons and Miss Sophia M. Simmons has yielded important information about this effect of iodine therapy upon the plasma "P" iodine. These investigators measured the "P" iodine before routine Lugol's therapy and at the end of a week's daily treatment with 15 minims of liquor iodi compositus (U. S. P.). In florid Graves' disease the plasma "P" iodine fell to normal values, or at least remained low. Recurrent, postoperative hyperthyroidism assumed a slightly higher level. Patients with toxic nodular goiter and normal individuals ranged higher, at about 30 to 50 gamma per cent. In myxedema very high values for plasma "P" iodine were sometimes obtained, e.g., well over 100 gamma per cent, although this finding was not invariable. In short, the level of "P" iodine attained in the plasma under Lugol's therapy appeared (as a crude approximation) to be a reciprocal function of the net thyroid activity. Control observations, in which iodide was added to the patients' plasma *in vitro*, showed no change in "P" iodine. (See footnote, p. 99.)

Thus even when thyroid activity is practically nil, a high concentration of organically bound iodine may accumulate in the plasma if large amounts of iodide are present. The findings warrant the search for some enzyme system, i.e., an iodase connected with thyroid function, such as Blum and de Quervain have postulated. The role of the thyrotropic hormone in this mechanism must also be investigated. (See footnote, p. 99.)

The source of the organically bound plasma iodine is obscure, but the following results show that serum protein itself can not be ignored. At the Thorndike Memorial Laboratory Drs. Albert H. Coons and Jytte M. Muus have iodinated horse serum albumin in stages. Up to an iodine content of about 5 per cent, i.e., while the constituent tyrosine was being iodinated, no therapeutic effect could be detected in human myxedema. Likewise, in this range no appreciable "T" fraction could be separated by alkaline hydrolysis. As further iodine was combined, however, thyroidal activity as tested in human myxedema became evident, e.g., near 7 per cent iodine. At this point a "T" fraction was easily separated. The potency of the iodo-albumin increased rapidly as more iodine entered the albumin molecule, and at 11 per cent iodine approached the maximal activity. Too large an excess of iodine altered the properties of the protein considerably, and simultaneously destroyed the endocrine activity. These findings raise still another problem. Is it possible that iodine can be bound in the "D" form in the absence of the thyroid, but that thyroid activity is needed for the conversion of "D" iodine to "T" iodine *in vivo*?

As Perkin and Lahey [433] have pointed out, "when the nature of iodine in the blood and the influence of iodine medication on the organic iodine components of *the* blood are more clear, a definite understanding will be attained regarding the iodine metabolism in diseases of the thyroid." This sentiment is especially cogent in clinical hyperthyroidism, because the hypothesis of Plummer keeps recurring in the literature, to the effect that in Graves' disease an abnormal hormone is elaborated. For example, in describing "The Mechanism of Secretion in the Thyroid Gland," Ludford and Cramer * concluded that "secretion in the thyroid gland in exophthalmic goitre differs . . . from that in the normal active gland. . . . The mechanism which controls the passage of the hormone into the circulation is no longer operative. If, moreover, the last-mentioned view is correct, the hormone which in exophthalmic goitre passes into the blood stream directly from the cell may be chemically different from that which in the normal gland passes from the alveolar lumen."

---

* R. J. Ludford and W. Cramer. Proc. Roy. Soc. *104*: 28. 1928–29.

It is clear from the foregoing confession of ignorance that there are great opportunities for investigation in the field of blood iodine and its fluctuations.* The chief impediment is no longer purely a lack of reliable micro-analytical techniques. The prime need at present is a better understanding of the qualitative nature of the iodine of the plasma. This problem will demand a considerable amount of skill in applied physical chemistry. For example, it was long held that the iodine of the plasma existed as a colloidal molecule, because it did not pass through standard dialyzing membranes. Recently, however, Perkin has found that when pure thyroxine (dissolved) is added to plasma, it likewise fails to appear in the dialysate. Such results indicate that the problem must be attacked in the light of modern knowledge of protein chemistry and, in particular, of the peculiar behavior of zwitterions. In the absence of such studies, this chapter must remain a simple record of present quandaries and obscurities.

---

* With regard to the high plasma "P" iodine observed after iodide therapy, it will be important to determine the type of combination by which the iodine is held to the protein coagulum. It might be (*1*) simple occlusion of iodide, in the presence of an excess of dissolved iodide; in other words, a technical artifact. It may possibly (*2*) represent a simple acid binding by protein. Finally, it may indicate (*3*) an organic combination of iodine. These possibilities remain to be explored. Because the phenomenon may be found in athyreosis, it merits careful scrutiny.

Likewise, further immunological studies should be made with partially purified plasma iodine fractions. In this connection it should be noted that the clinical observations of Lerman (cf. p. 75) have been confirmed in animals by L. I. Stellar and H. G. Olken (Endocrinology, 1940).

CHAPTER V

THYROID ACTIVITY

ALTHOUGH the thyroid is the chief regulator of iodine metabolism, it is not the purpose of this review to discuss the thyroid gland, its chemistry and physiology.[220] It must suffice here to consider certain quantitative aspects of its endocrine product, especially as it concerns iodine compounds and their calorigenic effect.

MANUFACTURE OF HORMONE

When elementary iodine or iodates are fed, they are converted into iodide before absorption.[91] Both iodine and diiodotyrosine can be absorbed from the intestine into the blood, and both can be removed from solutions perfusing the isolated thyroid gland. Although the gland may receive iodine from tincture of iodine painted on the skin or buccal mucosa [547] or by inhalation of ethyl iodide,[317] nevertheless the usual source for hormonal synthesis evidently is iodide in food or medication. Using radioactive iodide, Hertz, Roberts, Means, and Evans [241] recently have shown that the normal gland is surfeited with newly administered iodine within 15 minutes after administration. The newly trapped iodine is combined progressively with the thyroid protein, or else it diffuses back into the blood, so that after a day or two little inorganic iodide remains in the gland. Ultimately, only a small fraction of an ordinary therapeutic dose remains in the normal gland, although an iodine-starved gland may retain as much as 18 per cent of a single therapeutic dose.[352] With repeated doses the iodine content of the gland may be raised to over 1 per cent, dry weight. After such treatment, for example, the thyroids of rabbits contained 57 mg. of iodine, as compared with 16 mg. in thyroids of control animals.[374] Of course, organically bound iodine can also contribute to thyroid stores. For example, Klein, Pfeiffer, and Hermann [284] administered "jodtropon" for a week to hemithyroidectomized animals. Of 22.6 mg. of iodine so given, 5.1 mg. were retained, of which 1.9 mg. were recovered from the single thyroid lobe and 3.2 mg. from all other tissues.

The total iodine in the whole thyroid of apparently normal adult men may vary from 2 to 28 mg. of iodine.[139] In fresh tissue the iodine concentration varies from 1.1 to 166 mg. per cent (or from 12 to 431 mg. per cent in desiccated thyroid). Marine believes, however, that human tissue outside of the limits 0.1 to 0.55 per cent (by *dry weight*) should be considered abnormal. The difficulty in assessing normal values is enhanced by the fact that the iodine content varies with diet,[479] country, season, endocrinological balance and age. Furthermore, if iodine intake is low, not only is the content of the thyroid low, but compensatory hyperplasia of the tissue further reduces the gross iodine concentration and exaggerates the deficiency. Of the iodine so stored, normally about one-third is in "thyroxine-like" form in human glands, although in certain animals (e.g., Argentine sheep) this value may approach two-thirds. In other words, the major portion of the iodine incorporated in normal human thyroid protein has reached the diiodotyrosine stage, but has not been built up into the more complex thyroxine.* When the gland is secreting hormone at full capacity, its total iodine reserve may be depleted to less than one-tenth of the normal average. Furthermore, its thyroxine-like reserve may nearly disappear, presumably because it is removed as fast as it is synthesized.

Various estimates of apparent thyroxine in the whole human thyroid indicate consistently a store of from 3 to 5 mg.,[138] from 0.2 to 5.9 mg.,[315] or from 0.9 to 7 mg.[210] In dog thyroids, Elmer[138] found 0.7 to 1.1 mg. of apparent thyroxine, in rabbits only 3 to 5 gamma. The corresponding figures for diiodotyrosine are 2.0 to 6.1 mg. iodine in human thyroids, 0.2 to 1.19 mg. in dog thyroids and 4 to 18 gamma in rabbit thyroids. These latter figures include inorganic iodide, which is presumed to be negligible.

Topographical and Seasonal Variation. When the ingesta (including medication) contain much iodine, the thyroid traps large amounts, yielding a concentration exceeding 1.1 per cent by dry weight. On the contrary, if iodine intake is low the content of the thyroid is low, and compensatory hyperplasia of the tissue further reduces the percentage content. In mountainous countries the iodine content of the food is usually low, and even the air contains little iodine[168] at high altitudes. Here low values for the thyroid may occur. On the contrary, in maritime countries like Japan,[190] where fish and seaweed are consumed, the fresh thyroid may contain very high concentrations, e.g., from 79 to 109 mg. per cent of *fresh* gland (Mitsui, 1930). McClendon (1933) found that seaweed contains at least a thousand times as much iodine as any other form of food; indeed, one plant may yield 26 mg. iodine. In this connection the use of burnt sponge by the ancients for treating goiter is interesting.

---

* It is conceivable that a very small amount (2 per cent) of the iodine may exist in lipoid combination.[560]

In some parts of the world, like the British Isles, the time of year seems not to influence the thyroid iodine, as shown in Table 25. By some this has been attributed to a constant iodine supply from the sea. In the Mississippi Valley, however, seasonal variations are very important. Thus Kendall and Simonsen [277] found that the total iodine is highest in midsummer and lowest in February. Moreover, similar fluctuations occur in the thyroxine-like and diiodotyrosine fractions. The "thyroxine" fraction may practically disappear in February. Various explanations of this cycle have been advanced, among them the higher iodine in the diet in spring and summer and the greater secretion of thyroid hormone in cold weather, connected with changes of endocrine balance in the winter. Since the rise of iodine in the thyroid and likewise in the blood puts in its appearance in March, before iodine-rich foods are available, many investigators favor the latter explanation.

TABLE 25

IODINE IN DESICCATED THYROID GLANDS OF ENGLISH SHEEP (MARTIN)

1911	Per cent of dry gland
July	0.40
August	0.36
September	0.34
October	0.38
November	0.30
December	0.34
1912	
January	0.32
February	0.32
March	0.34
April	0.30
May	0.34
June	0.38

From: E. C. Kendall, Thyroxine, New York, The Chemical Catalog Co. (Reinhold Publishing Corp.) 1929. p. 55.

Bennholdt-Thomsen and Wellmann (1934) found that the action of ultraviolet light or the feeding of irradiated ergosterol can influence iodine metabolism. Their control rats, kept in the dark from February to June, showed a hyperactive thyroid with 211 gamma per cent iodine (dry tissue) and a low blood iodine (25 gamma per cent). The test animals, exposed to light or fed vitamin, showed a resting thyroid containing 112 to 167 gamma per cent, whereas the blood iodine was increased to from 31 to 40 gamma per cent. On the contrary, Elmer, Giedosz, and Scheps[144] were unable to change the aspect of the thyroid with vitamin D. Positive effects, however, were found by Nitschke (1933), who reported hypoiodemia in lack of the vitamin, and by Goormaghtigh and Handovsky (1935), who observed a thyrotropic action with small doses and a depression of the thyroid with large doses of the vitamin. In general, then, the cyclic variation in iodine seems to be the net result of a complex situation. (Cf. p. 191.)

As will be more clear from the chapter on thyropituitary interrelationships, seasonal or other changes in thyroid activity affect, and are reciprocally influenced by, the activity of other endocrine glands. Thus in the summer, when the sex glands of birds are hypertrophic, the thyroid is atrophic and contains little colloid. In hibernating animals, e.g., the hedgehog, bat, and marmot, seasonal changes are most marked. Their thyroids show regressive histological changes during hibernation, and an active histological picture in the summer. Indeed, thyroidectomized rabbits do not show an increased gaseous metabolism in summer, whereas normal rabbits show both increased gaseous metabolism and increase in total thyroid iodine (especially of the thyroxine fraction). Veil assumes that some unknown rhythm controls these variations; nevertheless it is clear that the endocrines participate in the cycle.

Ebb and Flow of Thyroid Iodine. The gland can regulate iodine metabolism[445] by fixing iodine and anchoring it to colloidal molecules, or by releasing such iodinated molecules or fragments thereof into the circulation. The action of the gland depends largely upon the properties of the blood which perfuses it. Foot, Baker, and Carrel[175] have studied isolated human thyroids preserved in the Lindbergh apparatus, and found that the final histologic picture depended on the nature of the perfusate, *not* on the previous condition of the gland. Likewise, Sturm[527] was able to study the result of various concentration gradients on the storage of thyroid iodine by working with isolated thyroids perfused with solutions containing various concentrations of iodide. With concentrations up to 500 gamma per cent of iodide in the perfusing fluid, the gland removes iodide from the arterial blood, thus producing a demonstrably lower venous concentration. Above a concentration of 500 or 600 gamma per cent, a short period of iodide retention is followed within 20 minutes by iodine elimination, so that the iodine level in venous blood is higher than in the arterial blood. Thus the gland's iodine reserve is reduced and organically bound iodine is released. This latter situation seems highly abnormal, and probably has little direct physiological significance, but it does demonstrate that the storage mechanism is reversible. It is puzzling that organic preparations like "jodtropon," or even thyroxine, when perfused through the isolated gland are not themselves stored, nor do they influence retention or release of thyroid stores.[527] Because this finding might suggest that iodine can enter the thyroid only in inorganic form, it deserves further study. Unfortunately, such data on diiodotyrosine are not available.

Sturm [527] also tested surviving thyroids from dogs which had been "satiated" with iodide for one or two weeks previously. These glands, when perfused with a solution containing iodide, quickly reacted by elimination of iodide! Three weeks after cessation of iodide feeding to the animals, the gland approached the normal reaction. Similar studies were made with glands from dogs which had been rendered thyrotoxic by administering "thyroidin" or thyroxine for several weeks. Such glands were atrophic, reduced to nearly half the normal size and showed flattened acinar epithelium lining follicles filled with colloid. Four such glands, however, reacted normally to perfusion with iodide by storing iodine. One other gland excreted iodine when the concentration in the perfusate reached 220 gamma per cent. Apparently the hormonal iodine, exogenous in origin, does not often yield enough iodide by decomposition to satiate the gland.

Regulation of Iodine Turnover. Besides delivering hormone to the tissues at large, the thyroid serves also as a reservoir for iodine, in which capacity it can regulate minor fluctuations in blood iodide. Perkin [432] and others have shown that in myxedema the blood iodine remains elevated longer than in a normal man after a single dose of iodide. Nevertheless, urinary iodide elimination is more rapid in myxedema (Courth [105]). Evidently the presence of the thyroid allows other tissues to fix iodide more effectively. Indeed, Maurer and Ducrue [374] found that the internal organs of thyroidectomized animals lose their power to store iodine.

In particular, when potassium iodide is administered, the response of the normal thyroid depends upon the route and duration of administration, the state of the animal, the dosage used, the species and possibly even the external temperature. The gland may be suppressed by doses below 5 mg. or by very large, continued doses. With regard to small doses, whereas 0.1 gram may produce 6,000 mitoses, only 200 mitoses appear after the administration of 0.01 gram.[336] After intraperitoneal injection a maximum effect may occur after 10 days, which is much sooner than after oral administration. After the cessation of iodine feeding, at the peak of the response, the thyroid returns to normal within a week.[446] Prolonged feeding of iodide for one month, however, may suppress thyroid activity, as shown in Fig. 11, rather than stimulate it. This is true even when large doses of iodide are administered.

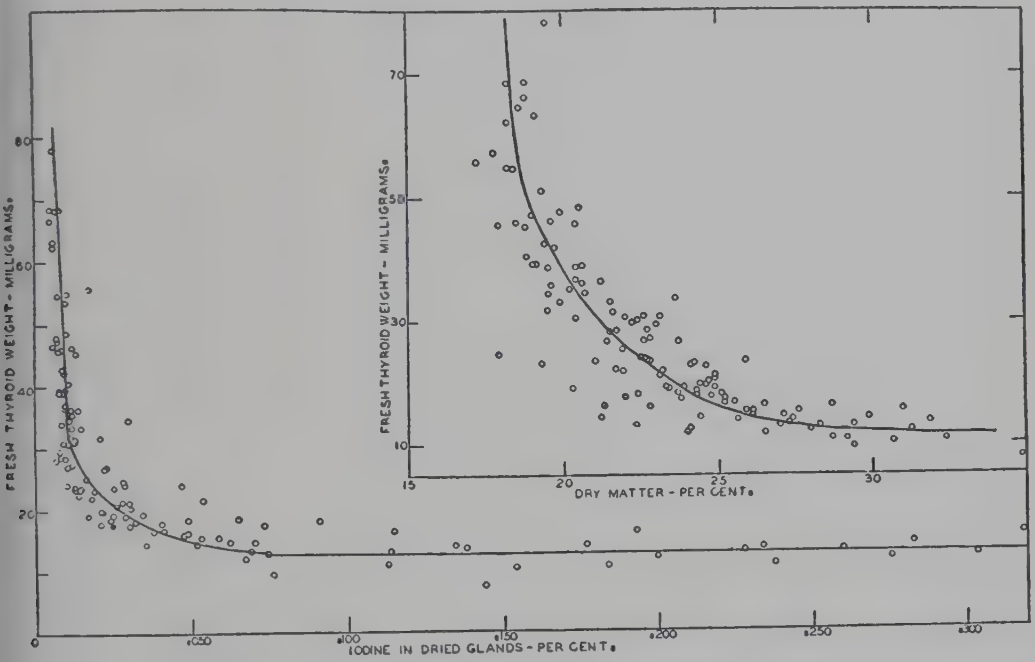


FIG. 11. The effect of iodine-adequate and iodine-deficient rations upon the thyroid glands of rats. The weight of the thyroids is given in milligrams per 100 grams of body weight.

From: R. E. Remington and H. Levine, J. Nutrition, *11*: 347. 1936.

When the gland is hyperfunctioning due to continued cold, iodide suppresses the mitotic activity, rather than exciting it as it does in the control animals.

Dogs, guinea pigs, and rats have been found to react differently to iodide. Thus Marine and Lenhart [360] found that iodide reduced thyroid function in dogs, whereas Gray and Loeb (1928) found stimulation in the guinea pig. Rabinowitch likewise found stimulation in rats, but with smaller dosage.[446]

Many authors have assumed that a gland showing active mitosis was necessarily actively secreting hormone into the blood stream. Siebert and Linton [508] found, however, that this is by no means the case. Normal guinea pigs show no appreciable rise in basal metabolism after treatment with iodide and thyroidectomized guinea pigs show a slight fall in tissue metabolism. The author has suggested [470] that the active thyroid gland may secrete internally into its follicles ("endocretion"), depending upon the polarity of the follicular cells.[505] The most striking example of this is the response of the gland to iodide therapy in exophthalmic goiter, as will be described shortly.

When diiodotyrosine was administered by Abelin and Wegelin,[10] the normal thyroid showed only flattening of the epithelium and accumulation of colloid; no stimulating effect was noted. Likewise, the thyroid hormone depresses mitoses to one-fourth the normal number, and "thyroidin" depresses compensatory hyperplasia after hemithyroidectomy. Loeb [331] found that this probably results from diminution of thyrotropic output from the pituitary. Indeed, Sturm [527] gave "thyroidin" to dogs over long periods and so produced atrophic, resting thyroids.

The ability of the thyroid to take up iodine depends in large measure upon iodine content.[558] Thus the nearly empty follicles in thyrotoxicosis testify to the avidity with which the gland removes iodide from the circulation.[566] Consequently, iodide fails to accumulate in the blood and the iodide tolerance curve is low, as pointed out by Elmer,[137] Perkin and Lahey,[433] and Fitz.[174]

Under these conditions inorganic iodide accumulates in the gland, and its transformation into diiodotyrosine begins under the influence of an enzyme system, "iodase," hypothecated by Blum and Grützner (1914). Gutman, Benedict, Baxter, and Palmer [210] described this process in the human gland, and noted progressive transformation of the diiodotyrosine into thyroxine-like material in a series of glands, illustrated in Fig. 2, representing the first week of therapy. Thereafter a stable composition of the colloid was found, indicating that iodine accumulated no faster than the normal composition of thyroglobulin would warrant, i.e., thyroxine-like iodide at one-third of the total iodine.

When the gland is not empty of colloid, or when large amounts of iodide are administered, the inorganic state persists in the gland, as found by A. Kocher [288] in man, and by Sturm [527] in surviving canine glands.

When large amounts of iodide are given, part of the inorganic iodide accumulated by the gland is turned back into the circulation and excreted.[241] The rest is transformed gradually into thyroid protein.

Endocrinological Regulation of Thyroid Activity. Although the question of endocrine balance is discussed in Chapter VI, two important points should be emphasized in relation to the control of thyroid activity. First, Loeb [331] found that "thyroidin" inhibited the action of thyrotropic hormone, probably per se and not through its iodine content. Its effect was more marked than that of inorganic iodine. Indeed, Gray and Rabinowitch (1929) found that "thyroid" alone checks normal thyroid function. In this respect the thyrotropic hormone may be said to produce its own antagonist.

Secondly, the effect of iodide on thyroid hormone is complicated. As stated elsewhere, the observations of Sturm [527] show that the thyroid itself responds to small doses of iodide by retention and to large doses by a sort of iodine-catharsis. Direct study of the thyrotropic activity of surviving pituitary tissue, however, also indicates that iodine can affect the anterior pituitary *in vivo*. Loeser [336] gave doses above physiological amounts, but nevertheless small from a pharmacological standpoint. He found the thyrotropic content of pituitary tissue diminished. On the contrary, it is clear that iodide does not prevent hyperthyroidism in man when the thyroid hormone is administered from an exogenous source.[503] Peripheral end-organ action, therefore, must often be very slight.

Daily Production of Hormone. On the basis of substitution therapy in human athyreosis, it seems clear that the human thyroid secretes about ⅓ mg. of thyroxine, or its equivalent, in 24 hours. On the basis of iodine balances, the optimal daily requirement is not less than 100 gamma iodine, nor more than 200 gamma iodine.[139, p. 277] Means [384] summarized his own data and the data of others, and reached much the same conclusion. Eppinger and Salter [159] confirmed his value by treating human myxedema with purified human thyroglobulin.

The amounts of hormonal iodine in the body can be estimated from the rate of decay of the metabolic rate in myxedema.[56] Such calculations suggest that, exclusive of the thyroid, an average human

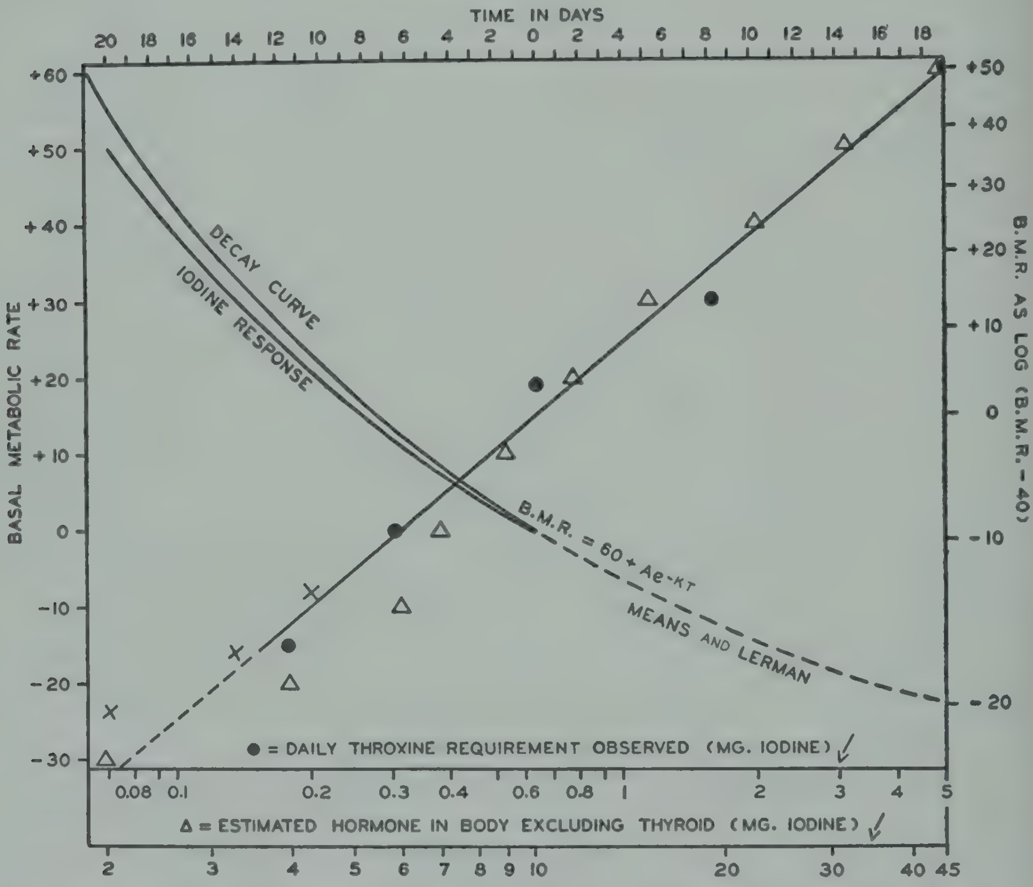


FIG. 12. Requirement of hormone with varying metabolism. Collateral data on a single individual, reported in part by Means and Lerman,[389] whose curves for iodine response and metabolic decay are reproduced. The straight line serves three purposes, provided the appropriate scales are observed: (*a*) with the right-hand ordinate and the topmost abscissa, the curve of Means and Lerman is illustrated as a logarithmic function; (*b*) with the left-hand ordinate and the lowest abscissa (open triangles), the hormone stored in extrathyroidal tissue is shown, as estimated by integration of the decay curve against time; (*c*) with the left-hand ordinate and the intermediate abscissa (solid circles) are shown actual observations by Lerman and Salter [319] of the requirement of thyroxine-iodine at several levels of hyperthyroidism. These data are supplemented by data (crosses) of Thompson [542] from another case recovering from myxedema.

From: W. T. Salter, Physiol. Rev. *20*: 365. 1940.

adult contains some 10 to 20 mg. of iodine, of which about two-thirds is in the form of thyroid hormone. These values are surprisingly consistent with actual analyses of tissues given elsewhere. The author and Lerman [319] have similarly observed the daily consumption of a single hyperthyroid individual at various levels of metabolism. This patient, previously reported by Means and Lerman,[389] was subsequently treated alternately with crystalline racemic thyroxine (intravenously), and with Armour's desiccated thyroid (U.S.P.) by mouth. Taken together with (*1*) the decay curve, and (*2*) the iodine response reported by Means and Lerman,[389] these additional data, — i.e., on (*3*) daily utilization at various levels, (*4*) differential effect of thyroxine as compared with whole thyroid, and (*5*) the theoretical hormone content of the body tissues, as estimated by integration of the decay curve against time, — all collected on a single individual, are extremely interesting because they supplement and reinforce one another.[468] Data on (*1*), (*2*), (*3*), and (*5*) are graphically presented in Fig. 12. The daily utilization (*3*), and the calculated content of the organism (*5*) both rise logarithmically with the basal metabolic rate. The dosage [319] required at a steady state of metabolism is shown in Table 26, together with comparative effects of whole thyroid and thyroxine. The desiccated thyroid, by analysis, contained 29 per cent of its iodine in the form of thyroxine. In the hyperthyroid range the actual thyroxine administered is more important than the total iodine in the thyroid substance used. This is not true in clinical myxedema [318] as explained later in this chapter.

TABLE 26

RESPONSE TO THYROXINE AND DESICCATED THYROID

Medication	IODINE IN SUBSTANCE USED (MG. PER DAILY DOSE)		Level of B.M.R.
	Thyroxine-iodine	Total iodine	
None	0	0	−15
Desiccated thyroid	0.17	0.6	0
$C_{15}H_{11}O_4NI_4$	0.5	0.5	+19
Desiccated thyroid	1.4	4.7	+30
$C_{15}H_{11}O_4NI_4$	4.7	4.7	+60

Genesis of Thyroidal Activity. There is now no doubt that the physiological equivalent of the thyroid hormone may be produced readily outside of the thyroid, as demonstrated by the data of

Lerman and Salter,[320] already described. Their observations raise the following questions: (*1*) does thyronine (thyroxine minus all four iodine atoms) exist preformed in serum protein as an essential amino-acid, awaiting iodination; (*2*) does the process of iodinating the protein also change molecular configuration so as to produce physiological activity; (*3*) can the thyroidless organism synthesize iodothyronine molecules from other iodinated residues?

The classical, rapid recovery of these individuals confirms Abelin's report [6] of the production of extrathyroidal hormone. Moreover, the fact that serum albumin, when simply iodinated, can completely relieve human myxedema casts doubts upon the prevailing conception that the gland's function is specifically to manufacture a hormone *de novo*. Lately, the synthesis from diiodotyrosine [75] has been conducted directly *in vitro*.[401] *In vivo*, Condorelli [100] found that dogs treated with 0.1 gram of diiodotyrosine daily remained healthy despite thyroidectomy. On the other hand, only feeble calorigenic action can be demonstrated when huge doses of diiodotyrosine are administered to human patients with myxedema.[539] There seems to be some peculiar function of protein combination, perhaps in orienting hydroxyl groups or in sealing off the amino-acid chains. Indeed, Cohn, Salter, and Ferry [93] noted a strange disappearance of tyrosine acidity on titrating iodoglobin. (Cf. P. Block, Jr., 1940.)

Recently, Ludwig and Mutzenbecher [344] have reported the isolation of thyroxine itself and of monoiodotyrosine from alkaline digests of iodinated casein and serum protein. Working with serum albumin, the author has obtained only a substance resembling diiodothyronine and a tetraiodo compound resembling deaminated thyroxine, but giving no Kendall-Osterberg reaction. This work is highly interesting, but as yet too recent to be evaluated.

Ordinarily, however, it is clear that the thyroid gland supplies the organism with the form of thyroid hormone which circulates in the blood. What this naturally occurring form may be is still a matter for conjecture, although the following observations on the calorigenic effect of various iodine compounds have definitely limited the possibilities.

Calorigenic Activity of Thyroid Hormone in Various Forms

There is little doubt that thyroxine [274] qualitatively reproduces the full effect of the thyroid hormone, because in human myxedema substitution therapy with crystalline thyroxine, judiciously administered, affords complete relief indefinitely. It should be emphasized further that the relief of human myxedema is the most reliable test available for thyroidal activity, and that results so obtained by experienced clinical investigators are more convincing than the measurement of oxygen consumption in animals or than metamorphosis experiments with tadpoles and axolotl.[554] This is especially true if quantitative observations lead to generalizations which are to be applied in the clinic.

Harington and Salter [229] isolated levorotatory thyroxine from thyroid protein. When the d- and l- forms made in Harington's laboratory were tested by Salter, Lerman, and Means [473] in human myxedema, no difference in activity could be discerned. In tadpole metamorphosis and in rat metabolism, however, Gaddum [191] found l-thyroxine to be thrice as effective as the d- form. This material also came from Harington. Similarly, Foster, Palmer, and Leland [179] found in normal guinea pigs that l-thyroxine is twice as active as dl- thyroxine; hence, they infer the d- form to be inactive, but no direct test of the d- form was made to prove it inert. The author believes that the difference in these results is due to biological dynamics, i.e., differences in rates of excretion, destruction and storage in various species receiving various dosages at different levels of thyroid function.

Thyroxine Derivatives. The high specificity of the chemical configuration of thyroxine was demonstrated [224] by synthesizing an isomer of thyroxine called "iso-thyroxine," namely, $\beta\beta$-di (3, 5-diiodo-4-hydroxyphenyl) α-amino-proprionic acid. Its proximate analysis and molecular weight are the same as the corresponding values previously determined for thyroxine. The two phenyl groups, however, are directly linked to carbon, whereas in thyroxine they constitute a diphenyl ether combination. Because this substance is inert, the thyronine configuration must be highly essential. Likewise, the iodine is highly essential because removal of the two (3′, 5′) iodine atoms on the outer ring reduces the activity to 4 per cent that of thyroxine. This diiodothyronine has been tested in human myxedema,[16, 321] and in animals.[191, 3, 448] When all four iodine atoms are removed it probably has no discernible activity.[191, 4] *

When all four atoms are replaced by bromine the resulting tetrabromthyronine has an activity only a fraction of a per cent of that of thyroxine.* When only the outer two halogens are bromine or chlorine, the inner two being iodine, an activity resembling that of diiodothyronine is obtained.[3] In tadpoles, thyroxamine, i.e., decarboxylated thyroxine, may have moderate activity,[3] although Harington [220] believes it to be inert. Further changes in the chemical structure of thyroxine cause practically complete loss of activity.

---

* On the contrary, Raab [444] thought he saw some decrease of metabolism in one of four cases of thyrotoxicosis. *In vitro*, curiously enough, Paal [424] found increased oxidation in isolated liver tissue treated with the iodine-free material.

There is disagreement as to the activity of esters and peptides of thyroxine. Harington [220] believes that the amino group need not be free, but Kendall disagrees. Likewise, Kendall [275] maintains that the acetyl derivatives of thyroxine have at best weak activity, whereas Gaddum [191] and Hoskins [253] found high activity both in diacetylthyroxine ethyl ester and n-acetylthyroxine.

Halogenated tyrosine may influence metamorphosis and the metabolism of amphibia. Difluortyrosine, however, is inert and may even antagonize thyroidal action.[328, 296] The dibrom-compound is essentially inert in tadpoles, but does stimulate the metamorphosis and gaseous metabolism of axolotl when given in large doses.[3] Diiodotyrosine causes tadpoles slowly to undergo metamorphosis, but its effectiveness is only 4 per cent that of thyroxine.

Although there is some evidence that diiodotyrosine has a mild calorigenic effect,[539, 448] the doses needed are huge and it will not relieve human myxedema. To be sure, it is highly effective in depressing basal metabolism in exophthalmic goiter, but no more so than an equivalent amount of iodide.[211] Likewise, it inhibits the action of thyrotropic hormone, as explained elsewhere.† Thus, although the suspicion is strong [223] that it is the natural precursor of thyroxine, this synthetic process has not yet been observed directly *in vivo*.

In summary, the high activity of thyroxine is connected both with its diphenyl-ether-alanine structure and also with its substituted halogens.

Several investigators [274, 441, 397] have maintained that thyroxine alone cannot be considered the only hormone secreted by the gland.

---

* Rapport finds that the effect on metabolism, referred to tyrosine, is as follows: tyrosine = 1; diiodotyrosine = 6 to 8; diiodothyronine = 7 × 25; thyroxine = 7 × 25 × 20 or 3500. See discussion by Kommerell.[294]

† This pharmacological action of diiodotyrosine is lost when the amino group is blocked by benzoylation.[515]

Laquer [307] and Abelin [7] also suspect the existence of other compounds. Lunde and Wülfert [348] tried to detect other iodine compounds (besides diiodotyrosine) in the gland without success. It seems likely to the author, on the basis of analogous experiments with iodized proteins, that the gland may contain 3,5-diiodothyronine, which would fall in the "thyroxine-like" fraction of Harington and Randall.[227] These latter authors believe, however, that within the losses of present isolation methods, which are admittedly inefficient, thyroxine and diiodotyrosine together could account for all of the organic iodine in the thyroid.

Artificial Iodoproteins. Lerman and Salter have recently tested the activity of artificially iodinated serum proteins, as described in Chapter III. Their data show thirteen successful responses in myxedema and cretinism and two successful responses in thyroidectomized rabbits produced by iodinated serum protein and its acid-insoluble degradation products. This material originated from the brief treatment of blood proteins with compound solution of iodine. In terms of total mass, the activity of the iodoprotein was about one-fifth that of whole thyroid; in terms of iodine, the activity was about one four-hundredth. On the other hand, the acid-insoluble iodopeptone was about half as active as whole thyroid in terms of total mass, but only one two-hundredth as active in terms of iodine. This discrepancy in activity is due partly to the presence of iodine in inert combination in the iodoprotein and in its derivatives. The low potency in terms of iodine is probably due also to the fact that the effective iodine-containing group is an iodothyronine derivative, which has lower potency than thyroxine.

Like thyroglobulin, this material can be fractionated with enzymes or by alkaline hydrolysis into thyroxine-like "T" and into diiodotyrosine-like "D" fractions. The latter were inert, the former possessed all the activity present in the original iodoprotein. As with the enzymatic split products of thyroglobulin, the D fraction yielded no thyroxine-like amino-acid after drastic treatment with hot barium hydroxide solution, whereas the T fraction yielded, under such treatment, material which resembled iodothyronine derivatives (related to thyroxine). The latter can likewise be recovered from the original iodoprotein by drastic alkaline hydrolysis without previous separation into component peptones. Such a procedure is the standard method for obtaining thyroxine from thyroglobulin. The activity of this iodothyronine material is of the order of crystalline diiodothyronine, and is roughly one-thirtieth to one-fortieth as active as thyroglobulin in terms of iodine. It corresponds to Abelin's "homothyroxine" material.

Recently, at the Thorndike Memorial Laboratory, Dr. A. H. Coons has tested the activity of iodinated serum albumin in human athyreosis. Such a preparation, made by Dr. Jytte M. Muus, has proved potent in myxedema when administered by mouth. Furthermore, it could be subdivided into T and D fractions, of which the T was active. This T peptone has been tested further by Dr. A. E. Meyer, through whose kindness the following results were obtained. When fed to rats for three days in a daily dose of 4.5 mg. per 10 grams of rat, this material produced a 30 per cent rise in metabolism, measured on the fifth day. This same metabolic increase could be reproduced by the daily administration of 0.75 microgram of commercial crystalline thyroxine per 10 grams of animal, or 300 micrograms of U. S. P. thyroid. Such therapy, however, would produce heart stimulation of 60 to 70 beats per minute or over 100 beats, respectively. Surprisingly enough, the iodopeptone increased the heart rate only about 30 beats per minute. For comparison, it should be noted that the normal heart rate of the male rat at one hundred days of age is about 300 to 325, whereas that of the thyroidectomized rat is only 185 to 200.

This untoward cardio-accelerating effect of whole thyroid suggests that special preparations like iodo-albumin or thyroid peptone may occasionally be preferred for therapy. This would be true especially in elderly patients or those with incidental, severe heart disease.

The Natural Hormone. There remain two puzzling facts with regard to thyroid activity which involve not only the true character of the natural hormone but also its pharmacological assay and dosage. The first fact, pointed out by Kendall, is that certain samples of desiccated thyroid yield no crystalline thyroxine although they are highly potent physiologically. The second fact is that desiccated thyroid or fresh thyroglobulin may produce more calorigenic effect than does the thyroxine contained therein.[31, 229]

In view of these facts, both Kendall and Harington have suggested that thyroxine itself is not the true hormone in its natural form. Indeed, Barger [29] suggested that the thyroid contained a more active substance than thyroxine, i.e., a compound which breaks down during isolation or is converted into the less active thyroxine. Kendall and Simonsen also speak of two hormonal forms: (*1*) thyroxine, as we know it, and (*2*) "active thyroxine." They suppose that the latter substance is the physiologically active molecule and suggest that it may be a hydroxy-thyroxine.[244] Thus, in tissues, compound (*1*) becomes compound (*2*) as the first stage of pharmacological metabolism. (Cf. findings of Davis and Hastings, 1936.)

Harington [221] suggests that the true hormone contains both thyroxine and diiodotyrosine, in peptide combination, connected by a link or bridge of other amino-acids. Nevertheless, neither thyroxyl-diiodotyrosine nor diiodotyrosyl-thyroxine, synthesized in Harington's laboratory, is as active as thyroxine itself.

Potency of Thyroid Preparations. The discrepancy between the

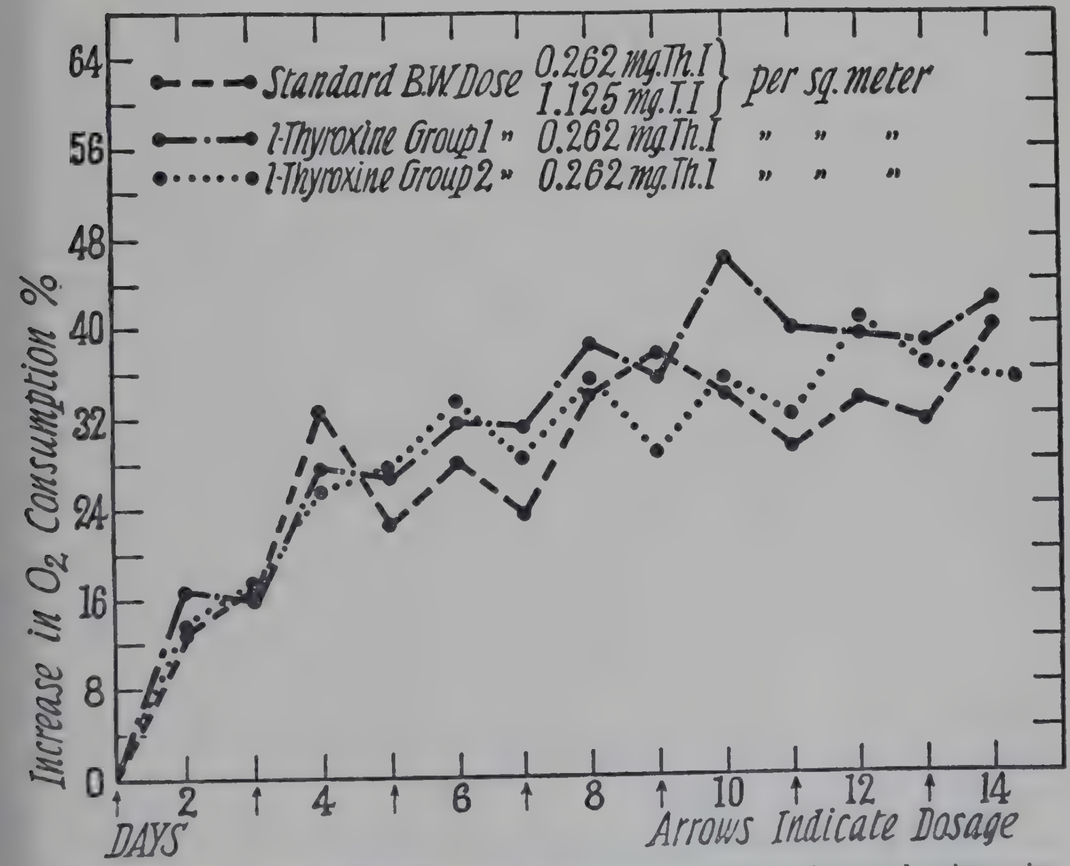


FIG. 13. Comparative responses in the oxygen consumption of normal guinea pigs produced by equivalent doses of a standard commercial thyroid preparation (Burroughs & Wellcome) and of pure l-thyroxine. Th. I indicates thyroxine-like "T" iodine; T. I. indicates total iodine. The dosage was calculated in terms of "T" iodine and the responses are identical.

From: G. L. Foster, W. W. Palmer and J. P. Leland, J. Biol. Chem. *115*: 475. 1936.

calorigenic activity of thyroxine and whole thyroid deserves special comment, not only because it is important pharmacologically and clinically, but also because it is still the subject of dispute.[541] Foster, Palmer, and Leland,[179] working with normal guinea pigs, found only the thyroxine fraction to be effective, as shown in Fig. 13. Likewise, Thompson, Thompson, Taylor, Nadler, and Dickie[545] found that desiccated thyroid on the average yielded only 62 per cent as much activity as intravenous thyroxine. They obtained variable results with different samples of desiccated thyroid.

On the other hand, investigators in Boston[390, 318, 473, 384] have treated several scores of cases of human myxedema with thyroglobulin and its various derivatives and find that, in proportion to the "T" iodine content, whole thyroid is distinctly more active in these patients than pure thyroxine, as shown in Fig. 14. Similar results have been reached by Freud and Laqueur,[184] using a digestion product of thyroglobulin, "thyranon." More recently, Meyer[393] has confirmed these results in rats and studied the effect of thyroidectomy.

> Male rats were thyroidectomized at about 80 days of age and allowed to progress into hypothyroidism for six or eight weeks. Their oxygen consumption was used as the criterion of the activity of thyroid medication, the dose of which was adjusted to produce an average increase of 30 per cent in metabolic rate. The normal rat proved to be too variable to serve as a reliable test object. Moreover, thyroidectomized animals were found to be about 25 times as sensitive to a given dose of thyroxine as the normal animal, because the former group required only 0.75 gamma of thyroxine to produce the standard rise in metabolism of 30 per cent. The data lead to the following conclusions.
>
> The thyroxine content of a given thyroid preparation does not permit a definite conclusion as to its calorigenic action. The iodine content seems to be a better guide, provided the substance has not been treated chemically, especially if it has not been subjected to hydrolysis.
>
> There is a species difference in the absorption of thyroxine through the intestinal canal of the human individual and the rat, respectively: within the experimental error, dissolved thyroxine is completely absorbed by the intestine of the rat.
>
> Isodynamic doses of various derivatives and preparations of thyroid have different but characteristic effects on the pulse rate of the thyroidectomized rat, ranging from a very slight increase (30 beats) to an increase of 175 beats per minute above the initial value of 180 to 200.[393, 394]

From his results it appears that the response at higher levels of metabolism is minimal and therefore unfavorable to fine discrimination of effectiveness. Thompson's data in man[538] are similar.

These various results indicate that natural combinations of thyroxine in peptide chains are more potent than their inherent thyroxine would be alone. Salter and Pearson[474] demonstrated this fact by breaking thyroglobulin down with pepsin and then resynthesizing a part with pepsin to form an artificial protein or "plastein." The

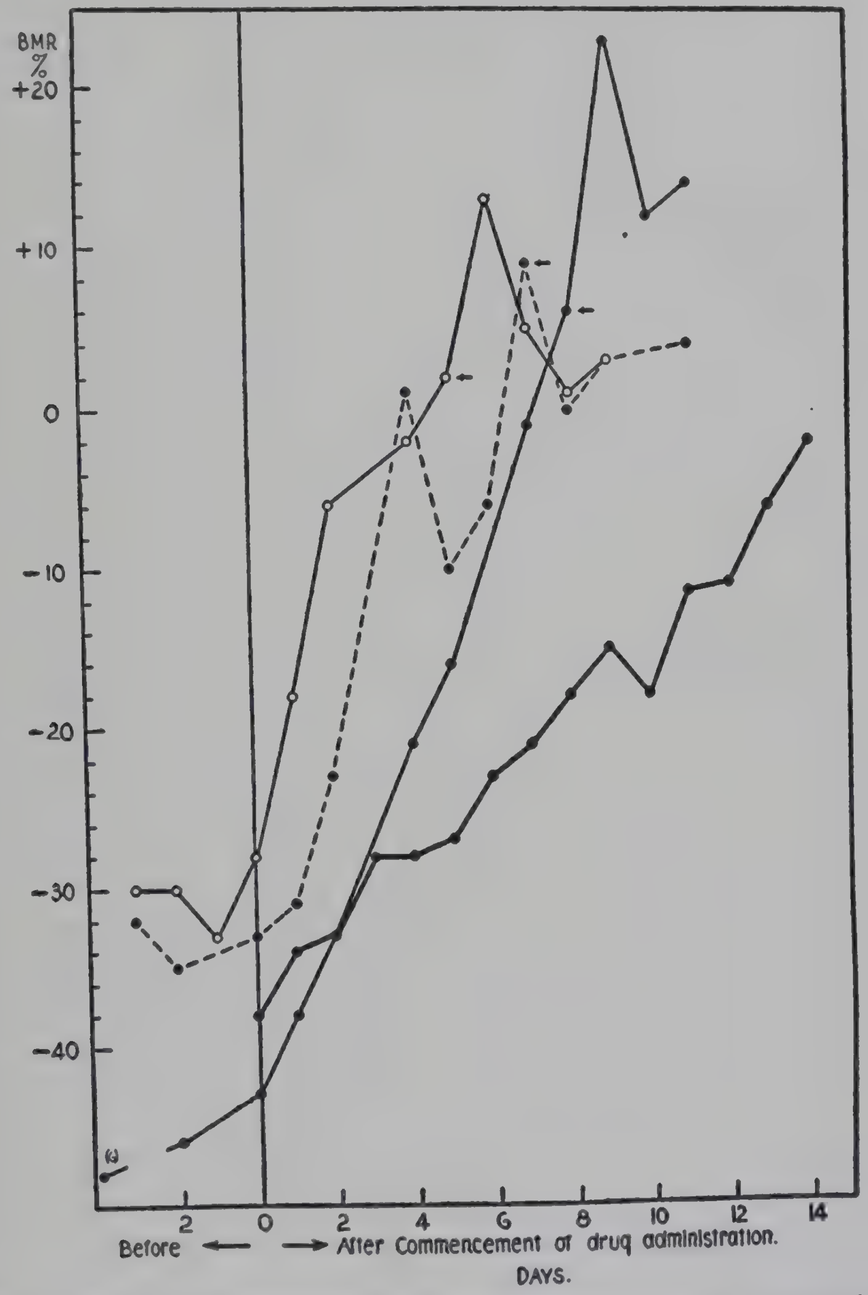


FIG. 14. The upper three curves are the calorigenic responses in three patients with spontaneous myxedema who received daily oral doses of thyroid gland containing 0.49 mg. of thyroxine iodine and 2.2 mg. of total organic iodine. The lowest curve, given for comparison, is the composite of five assays of thyroxine polypeptide given in daily oral doses containing 0.49 mg. of iodine. The small arrows on the upper three curves indicate cessation of medication.

From: J. H. Means, J. Lerman and W. T. Salter, J. Clin. Investigation, *12*: 685. 1933.

activity of the peptone (containing iodine chiefly in diiodotyrosine form) was increased sevenfold by the reverse synthesis.[470] Kendall[276] has suggested that the presence of the peptide chain prevents thyroxine from escaping (to the extent of 75 per cent) from the body, and thus increases its effectiveness. This explanation would relieve one of the embarrassments of explaining activity in diiodotyrosine. Clutton, Harington, and Yuill have prepared a synthetic combination of protein with thyroxine,[89] but its activity has not yet been assayed.

THYROID IODINE STORES IN DISTURBANCES OF THYROID FUNCTION

Obviously, the amount of iodine in the gland at any moment reflects the balance of intake over output. In disturbances of thyroid function the balance may be altered, and, secondarily, qualitative changes may occur in the nature of the stored colloid. These disturbances are usually comprehensible in terms of simple physiological mechanisms and may be summarized as follows.

Hypothyroidism. Diminished secretion of hormone from the thyroid gland may occur (*a*) because of a disturbance primary in the gland itself, or (*b*) because of diminished stimulation of the hypophysis through the thyrotropic hormone.[386] In the latter case, a normal amount of iodine remains in a shrunken gland.[256] Hence the average concentration rises 50 per cent (e.g., from 68 mg. per cent to 99 mg. per cent). Histologically, large masses of iodine-rich colloid remain surrounded by atrophic parenchyma.

Human myxedema usually implies nearly complete loss of thyroid tissue.[285] In cretinism, the gland persists but contains one-tenth of the normal amount of iodine.[465] Abelin and Finkelstein[8] studied the partition of the iodine in one such gland and found 57 per cent "thyroxine-like" iodine, as against 25 per cent for the normal.

Hyperthyroidism. In clinical thyrotoxicosis the iodine in the thyroid varies with several circumstances. Nevertheless, in untreated, moderately severe Graves' disease,[82] the iodine usually is low, due to depletion of colloid reserves, because clinical hyperthyroidism is based upon a hyperrrhea[220] of thyroid protein or its constituents. Indeed, in Graves' disease the concentration may be only about 1.2 mg. per gram of fresh tissue and the total thyroidal iodine about 5.8 mg., as against a minimum concentration in normal glands of 3.7 mg. per gram. By contrast, in mildly "toxic" nodular goiter the concentration may be increased to 19 mg. per gram of whole gland.[287] Unless iodine has been given, there is practically no inorganic iodine in the hyperactive gland.

The distribution of total iodine between cells and colloid is the same as in normal glands.[557] Because of the general use of iodine therapy preoperatively, recent observations are not available on the thyroxine-like fraction in marked hyperthyroidism. Wilson and Kendall[577] found that the thyroxine-like fraction tends to be less than the normal value for this fraction. Indeed, the relative amount is rarely over 16 per cent of the whole iodine, and in very severe hyperthyroidism it may all but disappear.[81, 178] Biological assays of the excised gland likewise indicate a low ratio of thyroxine iodine to total iodine.[301, 425] Chemical analyses show that the protein-bound iodine (i.e., the iodine in the form of colloid) is low, as one might expect from the histological picture.[347] Consequently, the diiodotyrosine fraction also drops. Wilson and Kendall's figures show, however, that the gland loses relatively less diiodotyrosine than thyroxine. This fact becomes comprehensible if one accepts the not improbable assumption that the hyperactive gland continues to make new hormone during hyperthyroidism, and it is able more easily to make diiodotyrosine than it is to complete the synthesis of thyroxine. Accordingly, relatively more of the former fraction persists. This view seems even more likely in view of analyses of the gland after injections of thyrotropic hormone into normal animals.[201] Under these circumstances the thyroxine:diiodotyrosine ratio remains normal despite considerable loss of colloid and of total iodine. In short, apparently both of the organic iodine fractions were lost together in this sort of temporary, artificial hyperthyroidism.

Wilson and Kendall[577] made parallel studies of the histological picture of the gland and its iodine content during exacerbations and remissions of the "primary" disease. In the active, hyperplastic stage the total iodine may drop as low as 0.03 per cent of dried tissue. In marked regressions it may return to 0.19 per cent, and simultaneously the thyroxine-like fraction may rise from about 10 per cent to 35 per cent of the total iodine.

Iodine Therapy and the Hyperactive Thyroid. The popularization of iodine therapy in exophthalmic goiter by Plummer[441] initiated a long series of speculations as to the mechanism of the striking temporary relief so afforded in most cases. In brief, the best explanation of the phenomenon at present is (the author believes) that the gland is made to secrete internally into its follicles rather than externally into the blood stream.[470] Thus iodide in excess may be said to reverse the direction of flow of hormone temporarily. (One is reminded of the reversing falls in the Bay of Fundy.) The salient facts upon which this statement rests are as follows.

1. Means [388] has shown that soon after iodide therapy begins, the basal metabolism drops sharply in inverse logarithmic fashion. The falling curve mimics that to be expected from thyroidectomy at a high level of basal metabolism, and can be extrapolated from the classical decay curve for approaching myxedema or cretinism, or after cessation of thyroid therapy in patients with myxedema [350, 56] (see Fig. 12). Means speaks of a state of thyroid block existing at this stage and thinks that iodide in excess always produces a resistance to exit of hormone from the gland. Indeed, the metabolism may drop to substandard, and possibly subnormal levels.[426, 383]

2. The hormone in the circulating blood apparently falls. The evidence for this statement is reviewed in a previous section.

3. The total iodine in the thyroid increases.[349] Were this increase all due to inorganic iodide, the fact would not be remarkable, but the organically bound iodine increases steadily with time.[210] In the earlier days of therapy, most of the bound iodine is in the diiodotyrosine fraction, and the thyroxine-like fraction comprises less than 18 per cent of the organic iodine. The conversion, or synthesis, into thyroxine-like material steadily speeds up, however, until after 10 days or a fortnight it catches up to its normal moiety, i.e., over 26 per cent of the organic. Thereafter, normal colloid seems to be laid down steadily unless further progress of the disease withdraws excessive amounts of hormone in the so-called "escape" phenomenon. This phenomenon has been discussed in detail by Means,[387] whose views are as follows: "In modern writings upon the action of iodine in Graves' disease one finds such terms as 'escape from iodine control' and 'refractoriness to iodine.' The former is clear enough and a good descriptive label for what happens when the administration of iodine is stopped in the thyrotoxic person who has previously been receiving it. Ordinarily a rather abrupt intensification of thyrotoxic manifestations takes place. Iodine apparently exerts its action in Graves' disease by imposing a resistance of some sort to

the delivery of thyroid hormone from gland to body. When the administration of iodine ceases, this resistance is removed and thyrotoxicosis is increased. Refractoriness to iodine would imply that although administration of the substance is continued, its ability to alter thyroid physiology becomes lost. That is to say that the patient becomes worse in spite of continued administration of iodine. It is certainly true that patients occasionally become more thyrotoxic while receiving iodine. It has been my experience that, even so, they still exhibit what has been described as escape when iodine is stopped. Their thyrotoxicosis is augmented at a still more rapid rate. Therefore, I feel that there is no refractoriness to iodine in an absolute sense, but I will concede that there may be in a relative sense."

In brief, then, iodine therapy in full-blown classical exophthalmic goiter converts the iodine-poor, colloid-poor goiter into one which is rich in iodine and distended with new-formed colloid. Salter and Lerman [470] have imitated this laying down of new thyroid protein *in vitro,* but it still remains puzzling how iodide (in excess) is able to reverse the flow of hormone. Our ignorance is due in part to lack of precise knowledge of how the intrafollicular colloid normally escapes into the circulation. One might suppose that reversal of the polarity of the follicular cells [505] is involved, but recently the existence of this phenomenon has been questioned.[386] At any rate, the beneficial effect of iodine seems to depend to a large measure upon the gland's being empty. Holst [251] compares the process to the filling of a bucket; when the bucket is full the hormone overflows and the so-called "iodine-escape" phenomenon ensues, clinically. This picture is not incompatible with Means's [388] view that, even so, iodine therapy always impedes the outflow of hormone in some measure. After prolonged treatment with iodide, the gland may contain much more total organic iodide and even more thyroxine-like iodine than normal glands. Histologically, the follicles are distended with new-formed protein. Even transplants show this.[570]

W. O. Thompson [540, 543] and his collaborators have found that a maximal therapeutic effect requires a minimal daily dose of from 6 mg. of iodine (in Boston) to 12 mg. (in Chicago). Obviously, this dosage is fifty times the amount needed to maintain iodine balance. Often clinicians routinely use doses many times this excessive amount, but such large doses are unnecessary.

When diiodotyrosine is administered, precisely the same result is achieved, in ameliorating clinical Graves' disease [294] and in increasing thyroidal organic iodine. Although on theoretical grounds [222] diiodotyrosine would be the more logical drug to use, in actual practice it offers no advantage over potassium iodide.[176] As with iodide therapy, only a small fraction of the diiodotyrosine is trapped by the thyroid, and if large doses be given much is excreted.[211] Whether inorganic iodide accumulates in the gland temporarily (as after iodide therapy) is not known definitely, but Gutman, Sloan, Gutman, and Palmer [211] recovered from such glands less iodide than in the ultrafiltrate from normal glands.

Other Effects of Iodine. It seems clear that iodide does not markedly neutralize the effect of exogenous thyroid in normal animals, because Segall [503] induced marked elevation of his own basal metabolism despite simultaneous iodide therapy, which had no effect on the metabolic level. This result has been confirmed by others in man [77] and in rabbits.[524] These observations also cast serious doubt upon a possible dampening by iodine of the responsiveness of peripheral tissues to the hormone.* There seems to be no question, however, that iodide can neutralize the effect of injected thyrotropic hormone. Indeed, Loeser and Thompson [339] insist that part of successful iodide therapy consists in suppressing the anterior pituitary secretion. Thus, in certain cases of clinical acromegaly the drug conceivably may both depress pituitary secretion and "block" the thyroid itself. Likewise, in experimental hyperthyroidism induced with thyrotropic hormone the administration of thyroxine inhibits the loss of thyroidal iodine.[332] Possibly whole thyroid substance has a similar effect in human hyperthyroidism,[457] though it may well be questioned how efficacious this might be as a general method of treatment.

"Jodbasedow" Effect. Although Means and Lerman [388] doubt the existence of Jodbasedow, i.e., hyperthyroidism in human individuals induced by iodine therapy, on the other hand many European clinicians, like Kocher,[289] fear to use iodine injudiciously. Furthermore, Webster, Marine, and Cipra [568] working with the cabbage goiter of rabbits, found that a temporary state of hyperthyroidism might be induced by large doses of iodine. Perhaps

---

* Siebert and Linton,[508] working with thyroidectomized guinea pigs, found that the response to thyroid was less in animals receiving iodide.

these views may be reconciled by the thought that a barely perceptible hyperthyroid trend in a time of iodine lack may become marked if iodide is supplied for conversion into large amounts of hormone. The problem is complicated, of course, by the cyclic nature of clinical hyperthyroidism and by the probability that the most dramatic effects of iodide therapy are seen when the gland is empty of thyroglobulin.

Simple Goiter. Most authors have found the iodine concentration to be subnormal in simple goiter, but the great variety of analytical results obtained in glands from euthyroid individuals suggests that more than one disease entity is involved. Since the total iodine content of these goiters tends to be normal, one would expect to find a subnormal average concentration, due to the increased mass. This is actually the case for, on the average, fresh goiter contains 7.4 mg. per cent iodine as against 13 mg. per cent in normal thyroid.[139, p. 463] (In dry tissue these figures become 44 and 64 mg. per cent, respectively.) There is a wide variation, however, namely from 8 to 376 mg. per cent in goitrous tissue and from 57 to 431 mg. per cent in normal tissue (dried).[210] Several factors contribute to this scatter. For example, it is not always easy to exclude therapeutic iodide intake or similar abnormal supply (e.g., painting of skin). Furthermore, the extremes tend to be colloid-poor and colloid-rich glands, and the iodine content in the colloid itself may vary. Many authors assume that the lower iodine concentration reflects a lower prevailing iodine supply, which induces hyperplasia of the tissue.[364, 456] Some other factor, possibly the degree of *relative* iodine lack, must intervene because Straub and Papp [522] found the same general concentration (15 to 16 gamma per cent) in normal thyroids, both from goitrous and non-goitrous regions. Elmer [139, p. 465] has suggested that two stages occur in the natural history of simple goiter. Because many patients fail to eliminate injected iodide normally in the urine, he believes that such cases represent hypertrophic glands with an increased capacity for trapping iodide. He believes that there is a second, later phase in the course of the disease in which the follicular cells become atrophic or degenerate.

In this latter type of gland the thyroglobulin is low in iodine, nearly free of thyroxine and inert when fed to myxedematous organisms.[318] In fact, the large follicular cysts are filled with useless colloid. In general, however, goiters which have not been too long neglected show a widely variable content, averaging close to the normal. Thus in a district showing a prevailing incidence of 15 per cent of goiter, the total iodine in the goitrous glands ranged from 2.3 to 6.0 mg., as against the average value of 5 mg.[139, p. 462] for normal glands.

In simple goiter there tends to be a rather low "thyroxine-like" iodine fraction, e.g., an average value of 14 per cent of the total.[136] In half of Abelin and Finkelstein's[8] cases in Bern, the average was only 10 per cent. Similarly, in euthyroid, nodular goiters collected in New York[210] and Minnesota,[81] the analyses range from 4 to 28 per cent. These values for apparent thyroxine resemble those found in hyperthyroidism, but in the euthyroid case they cannot mean excessive loss of thyroxine. They suggest rather that in simple goiter thyroxine is not readily formed. Perhaps the simple goiter may be regarded as being in a "virtual" hyperthyroid state, struggling to maintain normal metabolism on a deficient iodine intake.

It has been suggested that tyrosine deficiency also may be involved.[250] Nevertheless, Cavett[80] found the highest tyrosine content in glands containing the least "thyroxine-like" iodine.

CHAPTER VI

ENDOCRINE BALANCE

WHEN the thyroid is removed the high iodine content of the pituitary, as that of the ovaries and adrenals, drops to the level of other tissues (Sturm and Buchholz).[529] This observation suggests that in myxedema other endocrine organs besides the thyroid may be "sick" and underfunctioning. Because of the close relationship between the anterior pituitary and the thyroid, mediated through the thyrotropic hormone, this finding merits special attention. Further evidence of this interrelationship has been summarized by Elmer[139] in the following items: (*a*) the influence of the thyrotropic hormone on the histological aspect of the thyroid, its clinical picture, and the iodine level in the thyroid and in blood; (*b*) the action of iodine on the thyroid through the anterior lobe; (*c*) the effect of hypophysectomy on the iodine content of the thyroid, blood and urine;[302] (*d*) the influence of hypophysectomy on the iodine content of the tuber cinereum in the diencephalon.

THE THYROTROPIC HORMONE

Nearly a century ago Nièpce[410] noted that the pituitary in a goitrous cretin might attain a weight four times the normal. The significance of this hypertrophy became clearer 65 years later when Allen[13] and Smith[514] showed that the thyroid became involuted after the pituitary was removed. This involution could be reversed by implantation of tissue from the anterior pituitary or by injection of crude extracts of that gland. In rats, moreover, Foster and Smith[180] found that the basal metabolic rate decreased by one-third when the hypophysis was removed, but could be restored by injections of the pituitary hormone.

From these simple beginnings has come the long series of researches by the associates of Leo Loeb,[332] of Aron[18] and of Loeser[338] which have brought out the several features of thyrotropic activity. It is still debatable whether there is a natural hormone which has purely thyrotropic action. Perhaps this action is due simply to a single chemical grouping, one of several different, specific prosthetic groups borne collectively by a single protein molecule of pituitary origin.[98] * Most of the available evidence indicates that thyrotropic effects originate mainly and directly from the pars anterior, although Sturm and Schöning [531] have shown beyond doubt that an apparent thyrotropic action can be elicited by injecting extracts of the ovaries and adrenals. Indeed, Jensen produced thyroid hypertrophy with a purified luteinizing hormone preparation. Furthermore, Collip [98] has simplified the preparation of extracts showing specific thyrotropic activity by destroying preferentially such unwanted effects as gonadotropic activity.

Effect of Hypophysectomy. When the pituitary is removed the thyroid becomes smaller, but the iodine concentration increases. Houssay, Mazzocco, and Biasotti [256] explained these findings on the supposition that the colloid deposits remain, while the cellular elements atrophy. For two weeks after hypophysectomy the blood iodine rises from 17 gamma per cent to 52 gamma per cent, and drops slowly to 12 gamma per cent in the course of two months.† Simultaneously the basal metabolism falls to 80 per cent of normal, but the organism stops short of clinical myxedema. Sturm [528] has suggested that the transitory hyperiodemia might be due to stagnation in the venous circulation of the pituitary. At any rate, the final hypoiodemia is clearly due to depressed thyroid secretion. During the temporary hyperiodemia there is an increased urinary elimination of iodine up to 100 micrograms in 24 hours; that is, reaching some three times the normal daily amount. Later the iodine elimination becomes normal, with wide fluctuations due to diet.

Physiological Effects of Thyrotropic Hormone. Under the influence of the tropic hormone, reverse changes occur. If daily injections of a partially purified hypophysial extract are made intraperitoneally into mammals, definite enlargement of the thyroid follicular cells occurs and they continue to increase for several days. In larger doses this hypertrophy gives way to hyperplasia, associated with pyknosis of nuclei and with mitotic division of the follicular cells. Meantime the margin of the colloid

---

* Recent fractionation of the pituitary proteins in the laboratory of Professor E. J. Cohn, however, indicates that the several known tropic hormones appear separately in distinctive chemical fractions. (Endocrinology *26*: 999. 1940.)

† These concentrations are significant only in relation to each other. See footnote on page 71.

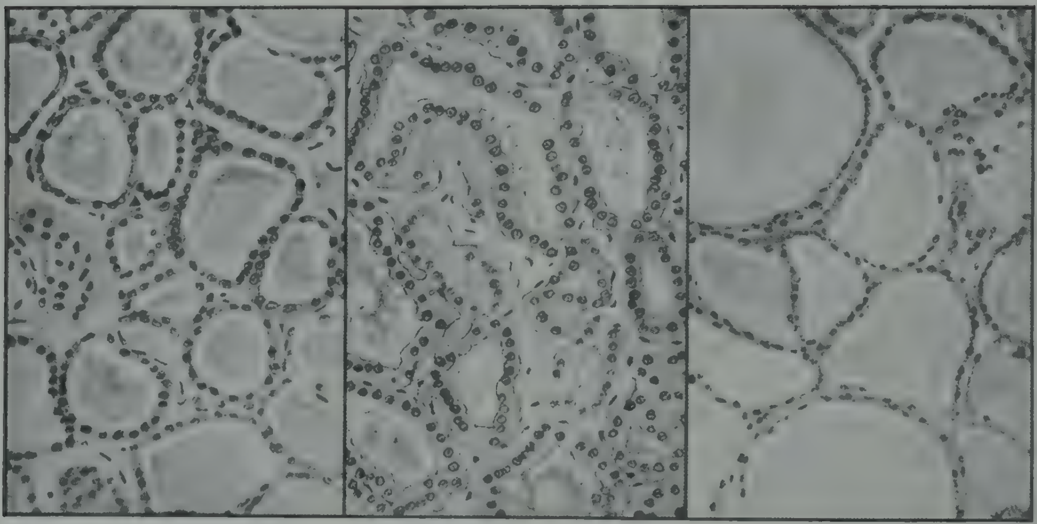


Fig. 15. Effect of extract of anterior pituitary on the rabbit's thyroid. At left, the thyroid of an untreated animal; in the center, the initial hyperplasia (resembling human exophthalmic goiter) induced by anterior pituitary; at the right, the ultimate involution, resembling human colloid goiter, as described by Marine.

From: S. Hertz and A. Kranes, Endocrinology, *18*: 416–418. 1934.

deposit becomes scalloped and, in stained histological sections, becomes less eosinophilic. The colloid gradually disappears as the follicular membrane becomes more and more hyperplastic and implicated, until eventually the gland bears a close resemblance to the hyperactive but depleted gland of human Graves' disease, as shown in Fig. 15. Under these circumstances, despite the loss of colloid, the gland increases in size and weight for a fortnight, due to increased vascularity and to increased mass of the parenchyma, which at this stage is found to be greatly enlarged with voluminous papillary infoldings. Under uniform conditions, these effects are so consistent and characteristic that they may be used for bio-assay of the amount of tropic hormone administered (cf. Appendix, p. 286). Moreover, Loeser,[338] and later Elmer,[145] showed that if increasing doses of the extract were used continuously, the effect continued until at length the animal succumbed to the classical sequelae of chronic thyrotoxicosis.

As these striking morphological changes occur in the thyroid under the influence of its specific tropic hormone, concomitant chemical changes take place which indicate profound alterations in metabolism of the gland.[15, 76] For example, Logan, VanderLaan, and VanderLaan [341] found that within twenty-four hours after the administration of thyrotropic hormone to guinea pigs, the water and chloride content of the cells increased, the colloid space of the gland decreased 50 per cent, and the oxygen consumption, per gram of tissue, increased 60 per cent. Continued administration for six days caused the gland to increase to three times its original size. The metabolism per gram of tissue and the chemical composition, however, tended to approach the resting values.

Meantime the "basal" metabolic rate, as closely as it can be measured, may rise to more than double its normal value, as shown in Table 27. The animal shows increased activity and excitability, with tachycardia and even cardiac arrhythmia. Marked loss of weight ensues despite an increased appetite at the outset. It is highly significant, too, that in certain species, notably birds and fowl, fish and guinea pigs, the animal becomes obviously exophthalmic during treatment.

It must be remembered, however, that when preparations other than extracts of the anterior pituitary are injected into normal animals, increased thyroid activity may occur. For example, Sturm and Schöning[531] found that extracts of ovaries and adrenals cause a strong thyrotropic action. A similar effect has been demonstrated[406] with crystalline sex hormones; this is possibly an indirect action involving the anterior pituitary. Schockaert and Foster[500] found little effect with gonadotropic hormone, a clear effect with growth hormone and a marked effect with thyrotropic hormone. These observations should be repeated with highly purified hormones, because later experiments indicate little thyroid effect with growth hormone.[162]

TABLE 27

EFFECT OF LARGE AND SMALL DOSES OF SODIUM IODIDE ON THE THYROTROPIC ACTIVITY OF AN ALKALINE EXTRACT OF THE ANTERIOR LOBE OF THE HYPOPHYSIS *

	PERCENTAGE OF ANIMALS SHOWING INCREASE IN BASAL METABOLIC RATE				
			Eleventh through fifteenth day		
Experimental conditions	First through fifth day, over +15 per cent	Sixth through tenth day, over +25 per cent	Over +30 per cent	Over +10 per cent	Under +10 per cent
Controls which received only injections of 2 cc. of anterior lobe pituitary extract	92.0	70.0	25.0	75.0	25.0
Guinea pigs which received injections of the extract plus from 0.1 to 0.2 Gm. of sodium iodide per kilogram of body weight	23.5	11.7	0	53.8	46.2
Guinea pigs which received injections of the extract plus from 0.0001 to 0.002 Gm. of sodium iodide per kilogram of body weight	72.2	61.1	38.4	84.6	15.4

* In addition to the more obvious differences in response elicited by large and small doses of sodium iodide, note particularly the unfavorable influence which the smallest doses of sodium iodide exerted on the course of hyperthyroidism induced by an extract of the anterior lobe of the pituitary from the eleventh to the fifteenth day of the experimental period.

From: H. B. Friedgood, Arch. Int. Med. *56*: 840. 1935.

At any rate, the effect of thyrotropic hormone is highly specific and definitive. The histological changes in animals described by Loeb[332] and by Aron[18] are easily reproduced. Furthermore, Schittenhelm and Eisler[490] have produced hyperthyroidism in man in four days by the daily injection of 1,000 (Schoeller's) units of thyrotropic hormone, and this finding was confirmed by Thompson.[546]

During this physiological response, the thyroid's stores of iodine are depleted,[500, 339] particularly the thyroxine-like fraction. Closs, Loeb, and McKay[88] found that the total iodine decreased by 45 to 79 per cent in guinea pigs treated for six days. Simultaneously the animals' blood iodine level increases. Schittenhelm and Eisler[490] verified this in man: they found a rise from 12 to 31 gamma per cent. This peak was not maintained, however. Zunz and LaBarre[588] found that the high iodine was largely organically bound, presumably in the form of thyroid hormone.

Parenthetically it should be added, although we are not greatly concerned with the fact here, that with small dosage of the extract the animal may become "refractory" in that it no longer responds to the medication. Indeed the thyroid may involute as injections are continued, and the basal metabolic rate may fall as much as 20 per cent below normal. This phenomenon has incited a great deal of work on the subject of antihormones, recently summarized by Collip.[99] The problem has been studied by Werner[573] and others, who have described the development of antibodies to the protein moiety of the tropic hormone. It is clear, however, that permanently hyperthyroid states may be produced by the continued administration of *large* doses,[338, 145] a procedure which obviates the depression formerly observed after two weeks of repeated injections. We shall avoid the question of an antihormone, raised by Collip and Anderson.[97] Various laboratories have lined up on the side of "antibodies" as against "antihormones," or vice versa. Because it is not yet clear that this interesting phenomenon has any direct bearing on natural processes, it will not be discussed further at this time. Antithyroid and antithyrotropic substances have already been described on page 93.

Many of the components of this complex physiological response can be attributed to the sympathetic nervous system. There is good evidence, however, that the effect on the thyroid can occur independently of nervous control.[423] For example, thyroid tissue transplanted into the abdominal wall responds characteristically when the animal host is injected with pituitary extract. Likewise Eitel, Krebs, and Loeser[134] showed that even surviving thyroid cells, cultivated *in vitro*, will respond to pituitary extracts; * and Foot, Baker, and Carrel[175] have observed a similar result when the whole thyroid organ is cultivated in the Lindbergh apparatus. In another chapter it will be necessary to discuss the relation of nerves to the

* Excellent pioneer work in this field was done by Okkels (1937).

complete picture, but at the moment we may be content to consider this thyroidal reaction as an essentially biochemical phenomenon.

Concomitant Fluctuations in Iodine. In terms of iodine, the striking histological changes just described may be followed by equally marked chemical changes, illustrated by Table 28. As the colloid in the gland disappears, a collateral loss of thyroid iodine occurs, until less than one-tenth of the original content remains.[88] At this point, scarcely a trace of the iodine remnant is in the form of thyroxine. Indeed, such glands, when excised, show almost no pharmacological potency.[301] The total iodine level in the blood may rise to three times the normal during the acute response, and, on fractionation of the total, the rise is found to occur chiefly in the organically bound "P" element. Further subdivision of this circulating organic iodine indicates that the "T" and "D" moieties are elevated in approximately the same proportions as those found in the resting normal colloid. In short, this phenomenon is extraordinarily like the "thyroid diarrhea" which Kocher described for human Graves' disease. Of course, a marked increase in urinary

TABLE 28

"ORGANISCHE" AND "ANORGANISCHE" IODINE IN THYROID GLANDS

Experiment no.	Group	Thyroid gland *	Total iodine		Alcohol-soluble iodine		Alcohol-insoluble iodine	
		mg.	mg. %	γ per gland	mg. %	γ per gland	mg. %	γ per gland
1	Control	23.6	30.2	7.2	1.0	0.2	29.2	7.0
	Injected	48.0	12.4	6.0	1.2	0.6	11.2	5.4
	Per cent change	+104	−59	−17	..	..	−62	−23
2	Control	29.0	33.0	9.6	1.6	0.5	31.4	9.1
	Injected	58.7	9.2	5.4	4.4	2.6	4.8	2.8
	Per cent change	+102	−75	−44	..	..	−85	−69
3	Control	28.7	27.4	7.9	0.6	0.2	26.8	7.7
	Injected	50.7	6.5	3.3	1.1	0.5	5.4	2.8
	Per cent change	+80	−79	−58	..	..	−80	−63
4	Control	28.7	27.4	7.9	0.6	0.2	26.8	7.7
	Injected	54.3	15.2	8.2	7.8	4.2	7.4	4.0
	Per cent change	+90	−45	+3	..	..	−73	−48

* Average weight per rat.

From: K. Closs, L. Loeb and E. M. MacKay, J. Biol. Chem. *96*: 590. 1932.

excretion of iodine occurs, with a striking negative iodine balance, which will be discussed in Chapter IX.

Such a situation obviously is pathological, but its importance lies in its heroic demonstration of the continual biochemical "tonus" to which the thyroid gland is subject. In old people rusticating in warm climates the degree of tonic stimulation is minimal, so that the human gland approaches the histological status found in the hypophysectomized animal as its basal metabolism sinks and the total metabolic requirement falls off. The sleek and sleepy pedigreed albino rat, living an Oriental carefree life in a well-regulated animal house, may present a thyroid which is "completely" resting. Such animals exhibit a very low endogenous iodine excretion, as might be inferred from their enforced inactivity.

When the struggle for existence is harder, however, the thyroid becomes "activated" according to the demands made upon the animal or man. As the pace of life increases, one may observe in sequence the series of events described above, appearing *gradatim* and *pari passu* with the stress involved. Extreme cold, sexual excitement, gestation and lactation, chronic fear, chronic depression of oxidation by cyanides, the need for food, — such are the circumstances which activate the thyroids of man and beast. Admittedly such phenomena operate through nervous channels, and these neurohumoral relationships will be discussed in Chapter VIII. For the moment, let us categorically assume that the final common path of these stimuli ends in the pituitary and increases its secretion of thyrotropic hormone, thus, at length, to release from the thyroid more of its stored hormone. This hypothesis is essentially that of Marine, who believes that thyroid hyperplasia and activity are regulated solely by thyrotropic tonus, as discussed in Chapter V.

The Pituitary-Thyroid Axis

Were not this regulatory mechanism delicately poised, excessive release of thyroid hormone could easily occur and in extreme cases the thyroid itself might become "burned out," i.e., become atrophic through cirrhosis. This result actually has been produced in experimental animals, and is observed in cretinism and in the clinical myxedema which sometimes follows neglected hyperthyroidism. Two factors operate, however, to oppose the release of pituitary hormone, namely, (*1*) the concentration of blood hormone, and (*2*) the concentration of blood iodine. The delicate balance so attained is another admirable example of the homeostatic mechanisms which, in Cannon's words, constitute the "wisdom of the body." Because this endocrine balance is of prime importance in the control of iodine balance, it will be profitable to examine it in some detail.

1. *Opposing Effect of Circulating Hormone.* Leo Loeb[330] injected a control group of guinea pigs with thyrotropic hormone and obtained the characteristic histological picture and metabolic response already described. Simultaneously he gave to a "test" group of guinea pigs not only the pituitary hormone but also a moderate dose of thyroid hormone in the form of thyroidin.* In consequence, the thyroid glands of the "test" group remained essentially unaffected. Similar experiments by Loeser and Thompson[339] and by Aron, van Caulaert, and Stahl,[21] performed with crystalline thyroxine, likewise indicate inhibition of thyrotropic activity by circulating hormone. When intact, normal animals are treated (*a*) with a certain dose of thyrotropic hormone alone, and (*b*) with thyroxine together with the same dose of thyrotropic hormone, careful comparison of these two groups shows that less thyroid hyperplasia results when thyroxine is given (group *b*) in addition to the tropic hormone. Similar results have been obtained with intact animals treated simultaneously with thyrotropic hormone and thyroid hormone in various other forms, namely, whole thyroid or derivatives of thyroid peptone.[333] Moreover, Kuschinsky found that the concentration of thyrotropic hormone in the anterior pituitary varies inversely with the extent of thyroid secretion.

In general, whenever the concentration of thyroid hormone in the circulating blood falls, that of the thyrotropic hormone probably rises. This is the so-called "castration phenomenon" which has been demonstrated even more clearly for the gonads than for the thyroid. In more precise terms, after the ablation of an endocrine gland which is normally under the influence of a pituitary tropic hormone, the pituitary becomes hyperactive and produces in excess the tropic hormone in question. This phenomenon has been clearly elucidated by van Dyke[559] and is well known to gynecologists treating patients with symptoms associated with the menopause. In the case of the thyrotropic hormone,[537] the evidence is less clear than for the gonads. Nevertheless it is possible to demonstrate increased thyrotropic activity of the urine in patients and in animals whose thyroid tissue has been removed in large part.[22] The same probably is true also in cases of spontaneous myxedema while the condition is still developing.[239] Increased thyrotropic activity of the blood, though likely in myxedema, has not been demonstrated so clearly.[102] Various assay methods are available for studying this problem, but they are crude and inconvenient at present. One of the more convenient procedures will be described in the Appendix. Already several biological "units" have been described, of which that of Schoeller[266] is perhaps best known. Most of these units depend upon the degree of histological change in the thyroid, but it is possible also to formulate a unit based upon how much the blood iodine may increase. Table 29 shows results obtained by measuring the height of thyroidal acinar cells in guinea pigs. The method is illustrated in Fig. 16.

---

* This indicates a peptone containing both thyroxine and diiodotyrosine in peptide combination. The term usually refers to desiccated thyroid.

TABLE 29

EFFECT OF VARIOUS URINES UPON THYROID CELL HEIGHT IN GUINEA PIGS *

No.	Group	Condition	Mean Height of Thyroid Cells	
			Range μ	Average
28	Control animals	Untreated	3.5 to 4.46	3.93
21	Normal	B.M.R. −9% to +11%	3.84 to 5.04	4.40
8	Hypometabolism	B.M.R. −17% to −34%	3.93 to 5.92	4.70
3	Hypothyroid	Total thyroidectomy	4.50 to 6.34	5.49
14	Hyperthyroid	B.M.R. +8% to + 90%	2.96 to 5.44	3.53

* Clinical studies with 50 cc. urine specimens.

From: P. Starr, R. W. Rawson, R. E. Smalley, E. Doty and H. Patton, West. J. Surg. *47*: 72. 1939.

2. *Opposing Effect of Iodine.* Because of the classic effect of iodine compounds in Graves' disease, much interest has been displayed in the influence of iodine compounds on this thyrotropic effect. Loeb showed that both potassium iodide and thyrotropic hormone may stimulate the thyroid (see Chapter V). Silberberg[510] found, however, that when the two are given simultaneously the result varies with the dosage of each used and with the time of administration. Therefore no single, simple statement can describe adequately the effect of iodide upon the balance between pituitary and thyroid. The difficulty lies in the fact that iodine affects both of these glands, and in addition may have some slight effect on the metabolic response of the tissues at large. Depending upon the mode of administration

and the respective doses of tropic hormone and of iodine, the latter may either enhance or inhibit the effect of the former. For example, Silberberg[510] injected guinea pigs intraperitoneally with a certain tropic extract every day for a month, giving 10 mg. of potassium iodide simultaneously. As judged by control animals who received no iodide, this drug increased the effect of tropic hormone. The extent of the synergistic action was greatest when small doses of iodide were combined with relatively large doses of hormone.

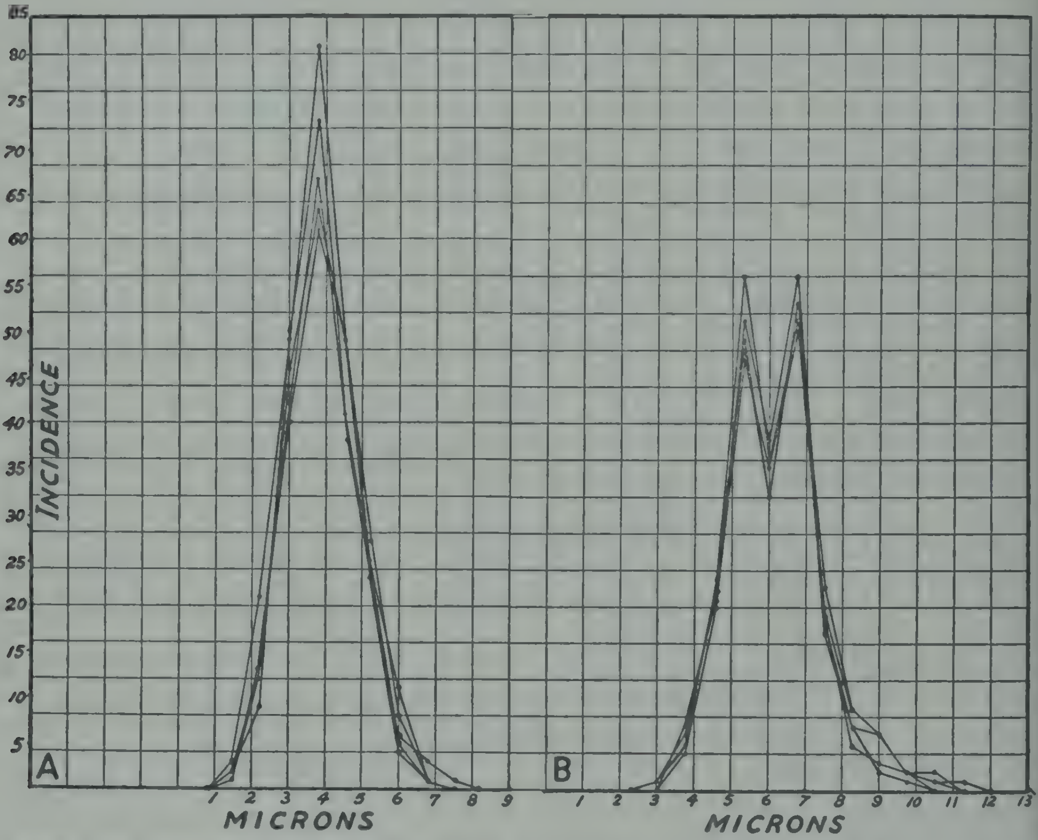


FIG. 16. Curves obtained by histometric measurement of two thyroids (five sections of each). (A) The gland of an untreated guinea pig. (B) The gland of a guinea pig after three daily injections of 0.02 cc. of thyrotropic hormone in the form of Antuitrin-T, each cubic centimeter of which contains 50 Junkmann-Schoeller units. From: P. Starr, R. W. Rawson et al., West. J. Surg. *47*: 67. 1939.

It is easier, on the other hand, to demonstrate that iodide depresses the tropic effect, as shown in Fig. 17. This fact has been shown admirably by many investigators, among whom are Siebert and Thurston,[509] Friedgood,[186] and Elmer.[145] The latter found, for instance, that daily doses of about 15 Schöller units of tropic hormone were largely nullified by the simultaneous dosage of 37 mg. of potassium iodide. How the iodine acts is unknown. It is thought to have three effects: (*1*) on the thyroid, (*2*) on the anterior pituitary and its thyrotropic hormone, and (*3*) on the end-organs peripherally. Salter and Lerman [470] have discussed the first of these as a mass

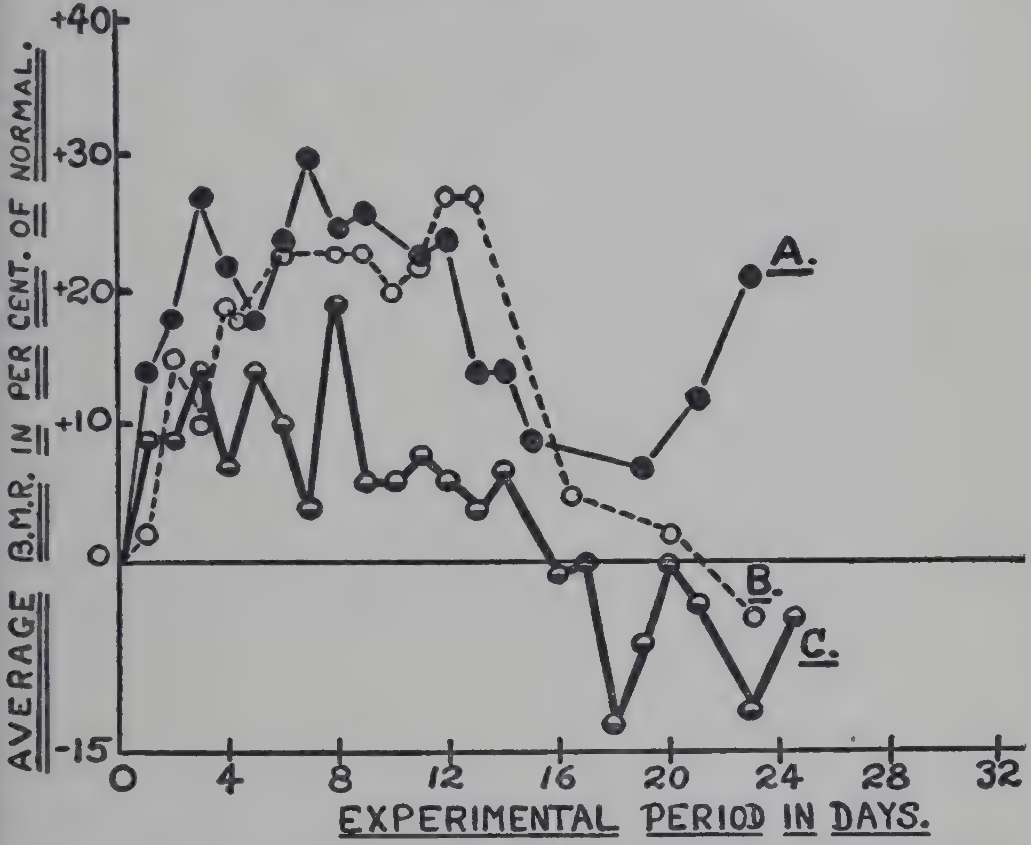


FIG. 17. The basal metabolic rate of guinea pigs injected daily with sodium iodide (in large and small doses) in addition to injections of anterior pituitary extract. The data were derived from averages of rather scattering results.

Curve A represents the averaged data on 25 control animals, each of which received daily intraperitoneal injections of an alkaline extract of the anterior hypophysis in doses of 2 cc. per kilogram of body weight.

Curve B represents the averaged data on 17 experimental animals, each of which was given the same dose of anterior pituitary extract as in A in addition to 0.0001 gram to 0.002 gram sodium iodide per kilogram of body weight.

Curve C represents the averaged data on 18 experimental animals, each of which received the same doses of anterior pituitary extract as in A in addition to 0.1 gram to 0.2 gram sodium iodide per kilogram of body weight.

From: H. B. Friedgood, J. Pharmacol. and Exper. Therap. *53*: 49. 1935.

action due to increased iodine concentration, which favors the storage of thyroid protein.

As might be anticipated from clinical experience, Abelin and Wegelin [10] found that diiodotyrosine exerted a similar inhibition of

TABLE 30

Effect of Iodide on Thyrotropic Potency of Rat Pituitary

Rat Donor			Guinea Pig Recipient		
No.	Preliminary treatment	Condition of the thyroid	No.	Condition of the thyroid	Number of implanted pituitaries
J 7	17 days on 0.05 grams NaI	In part active, in part filled with colloid	M 38	Active *	4
J 2		....			
J 5		....			
J 4		Inactive			
J 3	17 days on 0.05 grams NaI	In part active, in part filled with colloid	M 39	Active *	4
J 1					
J 8		....			
J 6		Inactive			
J 10	17 days on 0.05 grams NaI	In part active, in part filled with colloid	M 51	Active *	3
J 16					
J 17		Inactive			
J 14	17 days on 0.05 grams NaI	In part active, in part filled with colloid	M 52	Active *	4
J 9		Active			
J 19		In part active, in part filled with colloid			
J 11					
J 13	17 days on 0.05 grams NaI	In part active, in part filled with colloid	M 53	Active *	4
J 12					
J 15					
J 20					
J 29	5 days on 5 mg. NaI	Active	M 60	Very active	4
J 32					
J 30					
J 24					
J 31	5 days on 5 mg. NaI	Active	M 61	Very active	4
J 26					
J 21					
J 22					
J 28	5 days on 5 mg. NaI	Active	M 62	Very active	4
J 27					
J 23					
J 25					

* In these cases, especially marked cellular proliferation was noted.

From: G. Kuschinsky, Arch. f. exper. path. u. pharmakol. *170*: 529. 1933.

thyrotropic action. Indeed, iodine for iodine, it seems to be interchangeable with potassium iodide in this respect. These authors also obtained some effect with dibromtyrosine. Elmer,[138, 140] and Siebert and Linton [508] agree that diiodotyrosine is not superior to potassium iodide in its effect.

Several investigators believe that the effects of iodide upon the thyroid are always indirect tropic effects resulting from changes in the pituitary. Loeser and Thompson,[339] in particular, have estimated the content of thyrotropic hormone in the pituitary, and have found that very large doses of iodide lead to increased tropic potency, whereas very small doses depress the production of such hormone. Likewise Kuschinsky [306] injected large doses into rats and found a markedly increased pituitary content, as shown in Table 30. Diiodotyrosine gives a similarly variable reaction, according to the dosage employed. The actual range of daily dosage studied in rats was from 50 mg. to approximately 0.01 mg.,[306] but even the smallest dose exceeded by far the rat's daily requirement of iodine. These observations, therefore, are of great therapeutic or pharmacological interest, but do not lend themselves readily to physiological interpretation.

Indeed other workers, notably Marine,[355] have concluded both (*a*) that the only cause of increased thyroidal activity is increased tropic stimulation, and also (*b*) that pituitary activity augments progressively as the iodine supply approaches zero or, conversely, decreases as the supply of iodide becomes abundant. It is still a moot point whether this inverse relationship is maintained by the direct effect of the element on the hypophysis, or indirectly by an increase in circulating thyroid hormone, secondary to the abundant supply of *Ausgangsmaterial* (iodine) from which the hormone is manufactured. This point will be raised again in our consideration of the control of clinical hyperthyroidism in Chapter XI.

Pituitary Iodine. When the thyroid is removed the high iodine content of the pituitary, like that of the ovaries and the adrenals, drops to the level of other tissues.[529] The major part of this pituitary iodine is in organic combination, although little is known of the actual compound it makes. It is not even clear that this substance is very like thyroxine in its chemical behavior. Sturm and Schneeberg [530] reported that the normal anterior pituitary stored iodine as seen in Table 31. Thyroid feeding enhanced this storage, but the point requires further study. The total iodine concentration of the fresh mammalian anterior pituitary is about 80 to 190 gamma per cent. Similarly, Sturm and Buchholz[529] found in fresh human (whole) pituitaries 80 gamma per cent iodine, or 490 gamma per cent of dried substance. Closs[87] found in commercial desiccated anterior pituitary powder between 43 per cent and 96 per cent of the total iodine to be organic. Noether[411] studied the pituitary "colloid," in which he found about 1.3 mg. per cent iodine. This finding merits further study because it is denied by Baumann,[41] whose analyses are illustrated in Table 32.

TABLE 31

IODINE CONTENT OF DOG BRAIN (AVERAGE VALUES FROM 11 ANIMALS)

PALLIDUM		CEREBELLUM		MEDULLA OBLONGATA		THALAMUS + HYPOTHALAMUS + TUBER CINEREUM		HYPOPHYSIS	
Weight of brain analyzed in grams	Iodine content in γ %	Weight of brain analyzed in grams	Iodine content in γ %	Weight of brain analyzed in grams	Iodine content in γ %	Weight of brain analyzed in grams	Iodine content in γ %	Weight of brain analyzed in grams	Iodine content in γ %
22.15	6.41	14.4	9.03	10.0	8.7	1.95	27.69	0.38	486.84
22.15	6.32	4.8	9.37	10.0	9.0	3.9	25.64	0.19	494.74
Average iodine content	6.37	..	9.2	..	8.85	..	27.35	..	490.79

From: A. Sturm and R. Schneeberg, Ztschr. f. d. ges. exper. med. *86*: 670. 1933.

TABLE 32

IODINE IN HUMAN PITUITARIES

Case No.	Weight analyzed	I_2 PER 100 GM.		Remarks
		Wet	Dry	
	gm.	micrograms	micrograms	
374	0.33	<30	...	15–30 cc. cod liver oil daily for 1 yr. before death
555	0.24	<41	...	1 dose KI 2 days before death
604	0.30	<33	...	KI 3 weeks before death
368	0.30	150	660	Dressed with iodoform sponge in last wk. of life
371	0.45	80	390	Same
547	0.28	40	190	100 mg. KI daily for last 2 mos. of life

From: E. J. Baumann and N. Metzger, J. Biol. Chem. *127*: 114. 1939.

Pituitary-Thyroid Interaction. Thus there seems to be a constant regulatory relationship between the thyroid and the pituitary. In patients with the severe thyrotoxicosis of Graves' disease there is a decreased content of thyrotropic hormone in the blood and urine.[102] Conversely, in developing athyreosis Aron[19] found increased tropic hormone in blood and urine. As one ventures an appraisal of conflicting evidence, however, the major difficulty is lack of adequate quantitative control. Even in its simplest form, the pituitary-thyroid-iodine equation is tridimensional, whereas most of the observations available thus far control only one variable. In addition, the influence of time, including attendant fluctuations in concentrations and excretions, results in a devastating uncertainty. Henceforth, careful students of the thyroid-pituitary axis must measure (*a*) thyrotropic hormone in blood[170] or urine, preferably in both; (*b*) concentrations of the blood iodine fractions, "T," "D" and inorganic; and (*c*) some other variable or variables, e.g., basal oxygen consumption, metabolic iodine balance, or size of follicular cells in the thyroid. With these precautions, it should soon be clear how important each of the prime variables can be, and to this end the development of more useful assay methods must be pushed.[158]

In the meantime, some of these confusing data can be reconciled through a process of rationalization, which in retrospect will be called wisdom if correct and blindness if false. The high concentrations of thyrotropic hormone sometimes found in the pituitary may indicate a cessation of secretion, with resulting accumulation, rather than a greater outflow into the circulation. Similarly, the accumulation of acidophilic granules in the pituitary may reflect storage of hormone rather than increased output. Nevertheless, by analogy with the pituitary-ovarian axis, which has been exploited more intensively in various clinical institutes and physiological laboratories, one can piece together the fragmentary data bearing on the pituitary-thyroid axis as follows:

It is evident that the pituitary thyrotropic hormone is the chief method by which activity of the thyroid is ordinarily controlled. When the thyroid fails to put out sufficient hormone for the bodily needs, the concentration of tropic hormone in the blood is increased by pituitary secretion, presumably at the behest of nerve centers in the diencephalon. When the concentration of thyroid hormone in the blood becomes too high, whether from natural or from artificial causes, the pituitary ceases to provide stimulation and the thyroid assumes the resting state, histologically as well as physiologically.

The pituitary's response to environmental and to other chronic emergency states is remarkable. Among the situations which seem clearly to lead to increased pituitary thyrotropic activity are (*a*) thyroidectomy, (*b*) pregnancy and lactation, (*c*) growth, (*d*) continued cold, (*e*) paucity of iodine, (*f*) chronic infection, (*g*) indiscriminate administration of endocrine preparations, e.g., progesterone, (*h*) chronic poisoning with cyanide derivatives, (*i*) a continuous goitrogenic diet of such food as cabbage and (*j*) certain clinical syndromes,[101] which are disussed in Chapter XI. In most of these conditions the pituitary-thyroid interrelation is subject to change by the artificial alteration of the concentrations of circulating thyroid hormone or of circulating iodine from exogenous sources. In addition to the depressant effect of large doses of thyroid, which has already been mentioned, it must be remembered that small doses of thyroid may actually excite the pituitary. This form of therapy has long been used by practicing endocrinologists in adolescents having a tendency toward Fröhlich's syndrome. Rawson and Starr likewise found that in profound myxedema the pituitary may fail to put out thyrotropic hormone, as expected, until small amounts of exogenous thyroid have been supplied. Their findings are reproduced in Fig. 18. Better studies are needed to decide whether such inhibition of the pituitary merely checks the secretion of tropic hormone or actually inhibits its manufacture. In either case, concentrations of all three major variables must be borne in mind constantly. Thus there exists a delicate state of reciprocal stimulation and inhibition which usually results in very delicate balance or homeostasis.[21]

The precise details, however, are not clear. Does the tropic hormone merely accelerate the emptying of thyroid follicles or does it also directly speed up the manufacture of new hormone? Does the pituitary itself recognize the need for more thyroid activity, or do brain centers in turn regulate its activity? What is the exact nature of the circulating tropic hormone? Does it act catalytically in the thyroid tissue and, if so, how? These and similar problems offer a fertile field for further study. A good share of this pioneer work will lie in the field of neurological and psychiatric investigations, because it is already clear that neurohumoral mechanisms integrate endocrine and autonomic activity. Indeed, so close is the connection that often it is difficult to discover which system plays the dominant rôle at a given moment. Graves' disease is an excellent example of this confusion, as will be shown in Chapter XI.[516]

Thyroid and Other Endocrines

Because the activity of the thyroid is correlated[192] with that of other glands, one might expect that unusual states involving such interrelationships would affect iodine balance. The possible number of various combinations of hypo- and hyperfunction of one or more endocrine organs is enormous, but as yet the data are so scanty that one can cite only a few isolated instances which have been carefully studied from the standpoint of iodine metabolism. Thus far, there seems to be no clear-cut case in which such an alteration in iodine balance is not the direct consequence of altered thyroid activity, with the possible exception of the ovarian activity (to be discussed in Chapter VII) and also those disturbances which induce marked

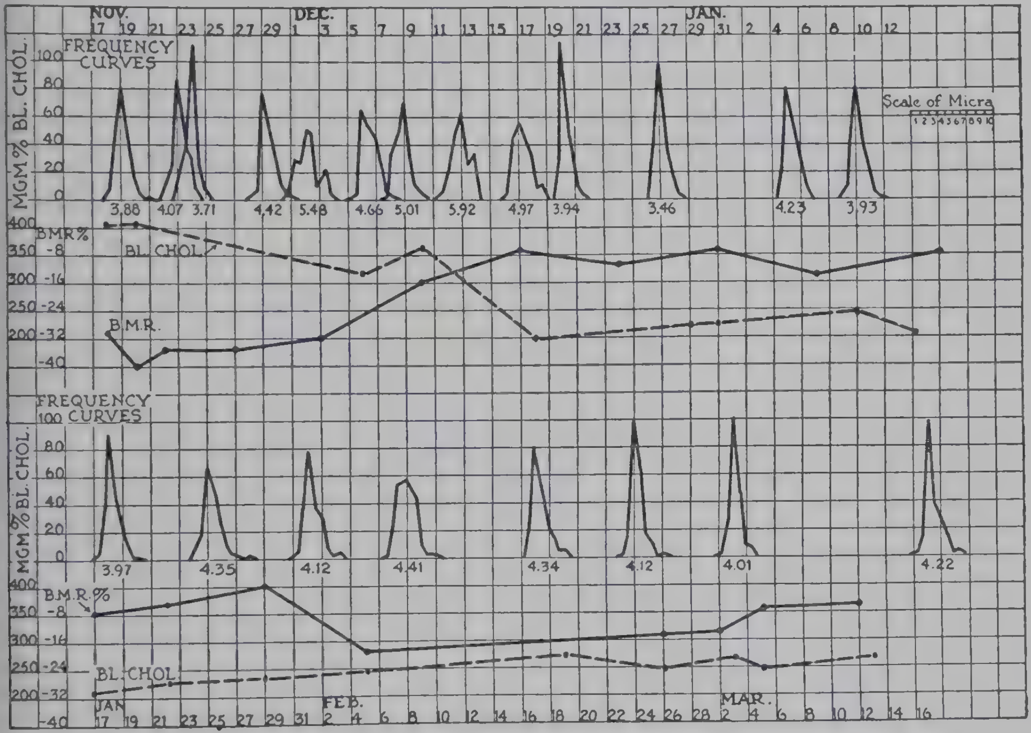


Fig. 18. Histometric assay of urine in spontaneous human myxedema before and after treatment. The assay curves are drawn to scale. The broken line indicates blood cholesterol; the solid line shows basal metabolic rate.

From: P. Starr, R. W. Rawson et al., West. J. Surg. *47*: 74. 1939.

wasting of general body protoplasm or at least of body water. A few of the better known reciprocities will be cited.

Adrenal Cortex and Iodine. Since the demonstration by Hoskins[255] that large doses of thyroid induce hypertrophy of the adrenal cortex in young guinea pigs, numerous observations have shown that there is an apparently antagonistic effect of the adrenal cortex on the action of thyroid hormone. When the adrenal cortical function is reduced experimentally in rabbits, many of the animals respond by an increased basal oxygen consumption which may endure for several weeks. This is obviously due to a thyroid effect, because it does not occur in athyreotic animals. Other animals, e.g., mice and white rats, respond to thyroxine more readily if the adrenal cortical function is low, and, conversely, increased cortical activity or concentrations interfere with the working of the pituitary-thyroid axis. Reiss and his coworkers[454] even reduced the blood iodine concentration, with concomitant involution of the thyroid, by administering adrenal cortical hormone in excess. Likewise several investigators[506, 290, 414] have found that cortical extracts afford partial protection against the metabolic effects of thyroxine and of thyrotropic hormone. After complete adrenalectomy, also, the expected life of the cat is shortened by administering thyroxine or, conversely, is prolonged by thyroidectomy.

Many of these findings can be explained on the assumption that a peripheral antagonism exists between the two hormones, which is independent of pituitary activity. On the other hand, it is difficult to avoid the conclusion that many of the associated phenomena involve either the pituitary-cortical axis, the pituitary-thyroid axis, or both axes simultaneously. For example, pituitary extracts fail to induce marked hypertrophy of the adrenal cortex in the absence of the thyroid and unless thyroxine be supplied. Similarly, in the presence of the pituitary, atrophy of the adrenal cortex or ablation of a major portion thereof leads to hypertrophy of the thyroid. Whether this thyrotropic action is an incidental result of a non-specific, "shotgun" *castration response*, or whether it serves some definite purpose is unknown. Nor have a sufficient number of data on the associated iodine balance been accumulated to permit of profound generalizations. In clinical Graves' disease, this interrelation may introduce an important complication, as described in Chapter IV and XI. (See footnote, p. 269.)

That the adrenal cortex participates in iodine metabolism might be suspected from its hypertrophy following injections of thyrotropic hormone.[335, 332] Treatment with iodide prevents this hypertrophy.[141] The problem needs more study because Marine [354] sees in thyrotoxicosis an adrenal hypofunction, whereas Crile [109] and Lewenfisz [323] see hyperfunction. No clear observations on iodine metabolism are available in Addison's syndrome or in adrenal hyperplasia, and these are sorely needed.

The adrenal medulla also shows a reciprocal relationship with the thyroid, but because this phenomenon is intimately bound up with neural status it will be discussed in a later chapter. With respect to the qualitative nature of adrenal iodine, it has been impossible to demonstrate any significant amount of "thyroxine-like" material in either part of the ox gland.

Thyroid and Parathyroid. In certain respects the parathyroid behaves like the adrenal cortex in its reaction toward thyroid activity. Large doses of thyroid extract induce hypertrophy of the parathyroids, while inducing simultaneously a greatly increased excretion of calcium. In hypoparathyroid animals, likewise, thyroxine may precipitate tetany. On the other hand, in rabbits which have been thyroidectomized the calcium turnover diminishes and the parathyroids may be found to be involuted. It was formerly believed that calcium metabolism might influence iodine balance. The results of Curtis and his associates and of Aub and his coworkers,[28] however, merely show that both iodine and calcium balances are altered secondarily in response to changes in endocrine activity.

Pancreas. The pancreas probably is involved in iodine metabolism only secondarily to its antagonistic action toward the thyroid.[143] Sweet and Ellis ligated the pancreas in dogs and found a marked increase in the colloid and iodine of the thyroid. After intravenous insulin, on the other hand, the blood iodine drops to one-half in an hour and a half [560] and remains at this level for some hours. Early in human diabetes Turner and Matthews [552] found that 27 per cent of their cases had values around 60 gamma per cent. Veil and Sturm [560] likewise found high values. Later, with poor nutrition, blood iodine may be subnormal. The gland itself has little predilection for iodine. In fresh whole pancreas Elmer [139] found from 13 to 20 gamma per 100 grams of fresh tissue. In dogs the total amount of pancreatic iodine is from 6 to 8 micrograms.[529] To what extent the islet tissue participates in the total is unknown.

Thymus and Thyroid. An antagonism between thyroid and thymus can be shown on several counts. For example, the feeding of thyroid regularly induces a rapid proliferation of the thymus cells, with hypertrophy of the gland in several species. Conversely, the thyroid involutes after removal of the thymus. Likewise, when an adult rabbit is treated with thymus extract, it responds less markedly to thyroxine than does the control animal receiving thyroxine alone. There is some evidence that this interrelationship obtains also in Graves' disease, a point to be studied further. It is not yet clear to what extent the pituitary participates in these phenomena. It is not certain, either, that the "alarm reaction" described by Selye has a definite clinical counterpart.

In summary, then, there is a dearth of strictly quantitative evidence as to the precise effect of the several endocrines on iodine metabolism. It is known that several of them limit thyroid activity and the need for thyroid hormone. Thus far they control the iodine turnover. Whether their antagonism is purely peripheral, or whether they influence directly the pituitary-thyroid axis has not been elucidated.

Peripheral Pituitary-Thyroid Relationships

Apparently distinct from the interplay of the pituitary-thyroid axis are other relationships which have to do with the response of peripheral tissues. For example, the pituitary growth hormone is effective in athyreotic animals,[162] but the simultaneous use of thyroid extract renders growth hormone more effective.[324] On the contrary, thyroxine fails to stimulate growth in young animals which have been both thyroidectomized and pituitectomized, despite its marked effect on the growth of animals and children suffering from simple athyreosis. Similar relationships which obtain between thyroid and ovary will be considered in Chapter VII, but it is clear that many peripheral hormonal responses become sluggish in the total absence of thyroid hormone. This observation is perfectly reasonable in view of the evolutionary fact that the thyroid was already functioning before the other endocrines appeared, as pointed out by Guernsey,[209] excepting the gonads.

A special case is the pituitary itself in total athyreosis, as already mentioned. The "captain" of the endocrine glands is dependent upon the thyroid for its own health. Accordingly, as myxedema develops in the athyreotic organism, the pituitary responds with increased tropic activity. Eventually, however, such activity ceases. It was once thought that the "castration phenomenon" had provoked such hyperactivity in the pituitary as to "burn the gland out" through overwork. It seems more likely now that the pituitary itself becomes ill and therefore ceases to secrete tropic hormone. At this juncture, if minute doses of thyroid extract or of thyroxine are administered, excessive thyrotropic activity may again be observed as the pituitary revives.[520] Pituitary iodine increases simultaneously from its low level, characteristic in myxedema. Only after more prolonged treatment with thyroid extract does the tropic activity cease because of increased circulating blood hormone. At the present writing this interpretation must be pieced together from fragmentary observation, with the better understood pituitary-ovarian axis as a guide.[189] Accordingly, it will be profitable to examine this other endocrine interplay for the light which it casts on iodine metabolism.

CHAPTER VII

IODINE AND THE PITUITARY–OVARIAN AXIS

Even among lay people, it has long been known that the thyroid is related to sex function, particularly in the female. It is common knowledge that the thyroid normally enlarges at puberty and, indeed, shows periodic changes during the course of the menstrual cycle. Furthermore, enlargement during pregnancy and lactation, and again at the menopause, is so characteristic that it needs no extensive comment here. Harington[220] has called attention to some rather amusing remarks by Benjamin Rush of New York in 1806, who said that the thyroid was a provision "necessary to guard the female system from the influence of the more numerous causes of irritation and vexation of mind, and the more acute bodily diseases to which they are exposed, than the male sex." Years afterward, in 1820, Hofrichter[249] retorted:

> Ist es nichts weniger als erwiesen, dass die Schilddrüse zu einer Zeit mehr Blut enthalte, als zu einer anderen — dieses würde der Augenschein lehren, und die Frauen würden schon längst die Mode mit entblösstem Halse zu gehen, verlassen, und die Männer schon längst das Schwellen dieser Drüse als Anzeige der von Seiten der schönen Hälfte drohenden Gefahr bemerkt und benutzt haben.

Even at the time of this metaphysical discussion, the tendency of puberty and of pregnancy to precipitate the development of goiter in regions which have a high incidence of endemic goiter had long been known. This finding is not true, indeed, in regions where goiter is almost pandemic, that is, in regions where the iodine supply is extremely low. In regions containing somewhat more iodine but still showing incidence of endemic goiter, however, the effect of sex becomes rather marked. Olesen and Taylor[415] have brought out this point by comparing goiter on the eastern seaboard (in Connecticut and Massachusetts) with that in Cincinnati and also with that in Minnesota. The seaboard states have a minimum of endemic goiter, Cincinnati has twice as much and Minnesota about three times as much. The extensive work of McClendon and his associates[377] has shown that these incidences correspond to different contents of iodine in the regions named. In these states the incidence of goiter in girls is definitely higher than that in boys. If one scrutinizes more carefully the group with moderate to marked enlargement, one finds that at the peak there are no boys in Connecticut and only 1 per cent of girls, corresponding to a reasonably adequate iodine supply. In Cincinnati, however, where the dearth of iodine is already beginning to tell, there are 20 per cent of girls but only 4 per cent of boys, namely, a ratio of five to one. Similarly, in Minnesota, where iodine lack is even more acute, 36 per cent of girls as against 6 per cent of boys are noted, that is, a ratio of six to one. Furthermore, it is clear that girls experience as much goiter at a low degree of iodine lack as boys do at a much higher degree of iodine lack. Likewise they tend to have larger glands than do those boys who develop goiters in the same environment. In view of the fact that the basal metabolic rate in women tends to be lower than that in men, this peculiarity is rather difficult to explain. In girls the development of goiter is more related to the onset of puberty than it is in boys. The extensive pioneer work of Marine [355] in the use of iodine as a prophylactic of goiter, one of the triumphs of modern medicine, illustrates clearly that iodine is perhaps the most important factor in this thyroid enlargement. In a recent article on the etiology of endemic goiter, Kimball [280] even goes so far as to say that this disease is one purely of iodine deficiency and therefore readily preventable.

Even with respect to exophthalmic goiter, statistics show an overwhelming preponderance of the female sex. For example, Bram [60] reports the incidence of Graves' disease as one boy to 20 girls in childhood, one man to five women in early adult life and one man to two women in elderly people. It is interesting to note the decline in the ratio as sex becomes less important in the life span. Indeed, when McCarrison [376] followed the "thyroid index," i.e., the ratio of thyroid weight to body weight, throughout the entire life of several species of animals, he found that the "thyroid index" became maximal just before the beginning of sexual maturity. Furthermore, Marine [355] has noted that his characteristic cycle, by which he explains nodular goiter, i.e., hypertrophy, hyperplasia and involution, is often precipitated by pregnancy. For these various reasons it is clear that in a thyroid clinic the women predominate to a marked degree.

Ovarian Iodine

Further evidence that the ovary is intimately concerned with iodine metabolism is derived from a comparative survey of the iodine content of various body tissues. Even in normal animals, Maurer[374] found that the ovary had the highest concentration of iodine in the body with the exception of the thyroid. He found a value reaching over 741 gamma per cent, although this figure is probably somewhat too high because Sturm and Buchholz[529] found values for human ovaries of betweeen 30 and 160 gamma per cent. Various values in the literature for animal ovaries also show rather high values, e.g., in dogs (bitches) 104 to 161 gamma per cent;[529] in hogs 609 to 648 gamma per cent;[529] in guinea pigs 340 gamma per cent;[529] in rabbits[371] 340 gamma per cent. Many of these figures have been reviewed by Carter,[78] who points out that in addition to the effect of iodine in the diet or ingesta, another significant factor which may influence the iodine in the ovary is its natural variation during the ovarian cycle. The effect of age was also studied by Maurer, Ducrue

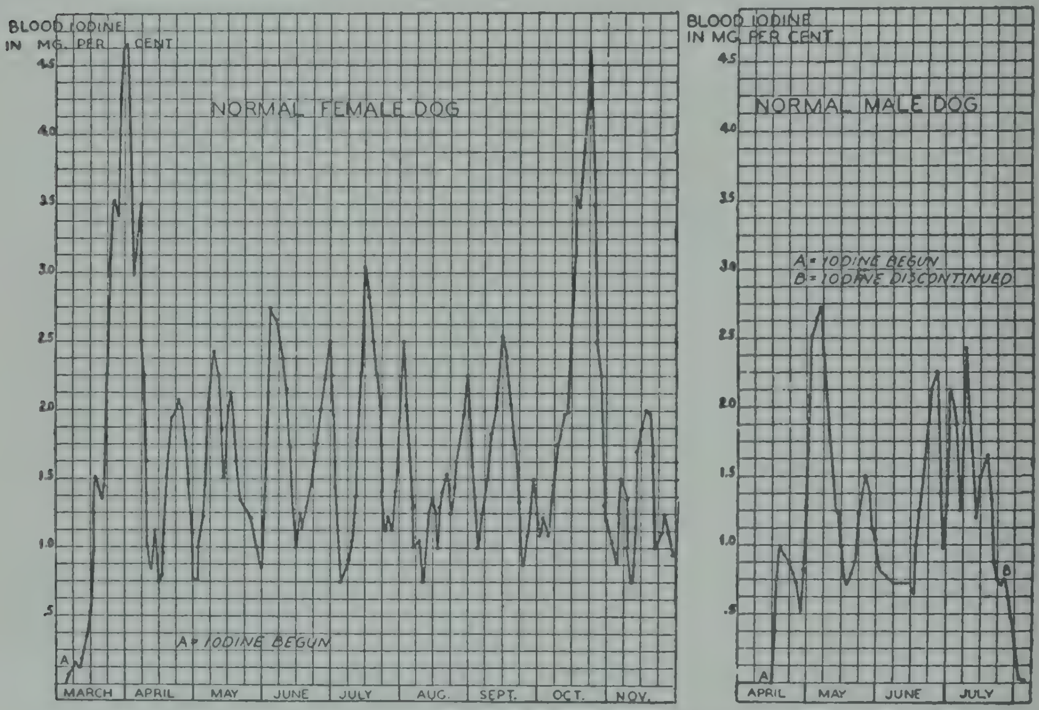


Fig. 19. Effect upon the concentration of total blood iodine of normal dogs of daily ingestion of a specific amount of iodine.

From: H. J. Perkin and B. R. Brown, Endocrinology, *22*: 539. 1938.

and Palasoff,[375] who found that ovaries in newborn babies averaged 138 gamma per cent as compared with adult ovaries at 741 gamma per cent. After the menopause, on the other hand, the ovarian iodine decreases.

When iodine, that is, iodide, is administered to dogs the importance of the ovary becomes even more clear, on comparing the male and the female, from the standpoint of blood iodine concentration. Perkin and Brown [429] studied the blood iodine of six female and four male dogs of the same breed and approximate body weight, i.e., 40 to 50 pounds. These animals were given 72 mg. of iodine each day in the form of compound solution of iodine and their blood was analyzed at frequent intervals over the course of several months. As shown in the accompanying Fig. 19, the concentration showed a series of peaks and dips varying from 75 to 2500 gamma per cent. Total removal of the thyroid in female dogs had practically no influence on the blood iodine, as shown in Fig. 20. Before thyroidectomy the blood iodine was nearly 2.0 mg. per cent and after thyroidectomy it was at least 1.5 mg. per cent. By contrast, after removal of the thyroid from the male dogs, the level of blood iodine was considerably depressed, i.e., from about 1.5 mg. per cent to 0.5

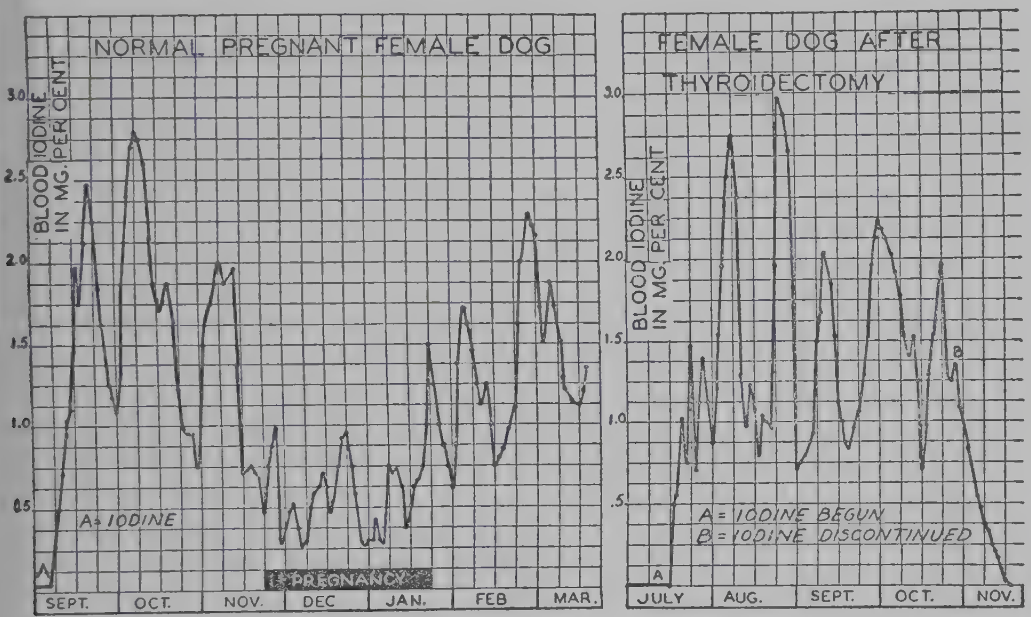


FIG. 20. On comparison with the preceding figure, thyroidectomy can be seen to have influenced the blood iodine but little, under the circumstances described.

From: H. J. Perkin and B. R. Brown, Endocrinology, *22*: 540. 1938.

mg. per cent. Of course these levels are far above those to be expected from a normal diet, and the blood level shows considerable fluctuation through the course of the day. Nevertheless, the charts show a very clear trend and a general mean level about which the blood concentration fluctuated. When these thyroidectomized bitches, however, were subsequently subjected to bilateral removal of the ovary, the blood level decreased markedly, as shown in Fig. 21. Indeed it dropped to about 0.25 mg. per cent, a concentration slightly under that of the thyroidectomized males. It is difficult to escape the conclusion pointed out by Perkin and Brown that in the female dogs the presence of the ovary was able to compensate to a considerable extent for lack of thyroid.

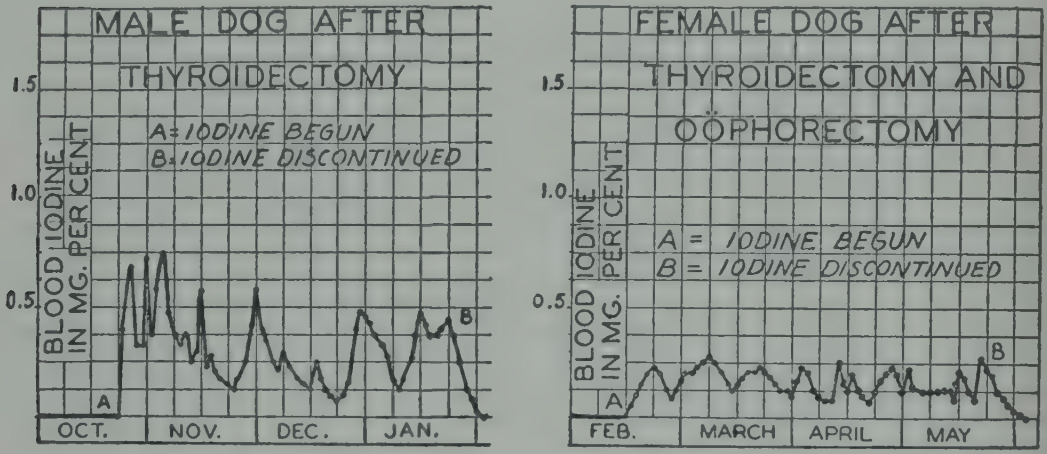


Fig. 21. A marked change in blood iodine (under the circumstances described) occurred in the male after thyroidectomy, but not in the female until both ovary and thyroid were extirpated. For comparison with Figs. 19 and 20.

From: H. J. Perkin and B. R. Brown, Endocrinology, *22*: 541. 1938.

When the iodine content of all the tissues of these animals was analyzed, as shown in Table 33, it became clear that, next to the thyroid, the ovary had the highest concentration of any tissue.

As regards the qualitative nature of the iodine in the ovary, further work is needed. This problem was attacked by Carter,[78] who studied the ripe ovaries of Echinus esculentus. He applied the method of Harington and Randall [227] to the tissue and was unable to detect any considerable "T" fraction. The percentage of apparent thyroxine-like iodine actually was only about 0.011 to 0.017 per cent of crude thyroxine-like material. On the other hand, with an extract of the organ, he was able to influence definitely the metamorphosis of tadpoles. The possibility remains, therefore, that even if thyroxine is not present combined in the tissue as such, a derivative of thyroxine may be present. Inasmuch as the ovarian iodine tends to fall after thyroidectomy, it would be reasonable to guess the ovary contains a specific form of iodine related to the thyroid which it extracts from the circulation.

Ovarian Cycle and Iodine Metabolism. The concentration of blood iodine has been studied, through the ovarian cycle, by several investigators, among them Jahn and Kesselkaul [263] and Leipert.[310] In general, there seems to be a definite rise in blood iodine at the onset of menstruation, amounting to about 25 or 30 per cent of the normal. Occasionally the absolute level may exceed 20 gamma per cent, but this is unusual. This rise is variable; the level on the average is 20 or 25 gamma per cent (Scheringer),[485] although Maurer and Diez [372] and Leipert [310] have found values as high as 110 gamma per cent. At the same time urinary iodine excretion is increased (Cole and Curtis),[95] and the basal metabolic rate rises. As described in another chapter, this increase may be more than double in women suffering from thyrotoxicosis, but apparently it does not occur in neurasthenia or related conditions. As found by Jahn and Kessel-

TABLE 33

IODINE CONTENT OF TISSUES OF DOGS RECEIVING IODINE DAILY

	MG. OF IODINE PER CENT (WET TISSUE)			
	A[1]	B[2]	C[3]	D[4]
Thyroid	18.3	27.6	31.2	34.6
Kidney	0.21	0.31	0.35	0.43
Liver	0.16	0.42	0.38	0.33
Lung	0.21	0.34	0.29	0.37
Heart muscle	0.24	0.33	0.32	0.30
Adrenal	0.19	0.52	0.41	0.29
Ovary	0.43	0.66	...	0.63
Testis	...	...	0.36	...
Bile	0.20	0.28	0.27	0.26
Blood	0.24	0.36	0.42	0.46
Feces	0.20	...	...	...
Intestine	0.18	...	...	...

[1] Animal A received iodine daily for 1 month.
[2] Animal B received iodine daily for 4 months.
[3] Animal C received iodine daily for 7 months.
[4] Animal D received iodine daily for 12 months.

From: H. J. Perkin and B. R. Brown, Endocrinology, *22*: 542. 1938.

kaul,[263] the menstrual blood may contain from 42 to 113 gamma per cent, possibly through concentration of the escaping fluid. Before menstruation ceases the blood iodine concentration becomes normal, and is maintained at a normal level until menstruation again occurs. At the onset of the menopause there is a tendency toward an increased blood iodine concentration, as described by Cucco.[110] He found that there was a slight increase in blood iodine early in the menopause, which tended to subside after several months. That is, whereas his level for women between 20 and 35 years of age was less than 10 gamma per cent, 36 out of 40 cases at the climacteric had values ranging from 10 to 22 gamma per cent. He found, furthermore, that after the injection of ovarian extracts for two or three weeks the blood iodine level might drop from about 20 gamma per cent to about 15 gamma per cent, i.e., his normal value. By contrast, cases without any increase in blood iodine above the normal level failed to show any effect from ovarian extract. Within a few months this slight rise in blood iodine had disappeared. Indeed, if one finds abnormal blood iodine after approximately six months, it is suggested that some other condition exists. Likewise, Jahn and Kesselkaul [263] found an average increase in blood iodine of about 15 per cent above the normal during the first year of amenorrhea.

TABLE 34

THE BEHAVIOR OF BLOOD IODINE FOLLOWING OVARIECTOMY IN YOUNG AND OLD BITCHES

	Blood Iodine, γ %				
	Before ovariectomy	After ovariectomy			
		1 week	2 weeks	3 weeks	4 weeks
Bitch, young 7.8 kg. . .	10.0	20	11	8	11.5
Bitch, young 7.3 kg. . .	8.5	20	6.5	7	7.5
Bitch, old 15 kg.	12.8	13	7	8	9
Bitch, old 10 kg.	12.8	13	13	8	6.5

After: M. Franke and L. Ptaszek, Compt. rend. Soc. de biol. *114*: 1129. 1933.

It is altogether likely that this menopausal increase in blood iodine reflects increased activity of the thyroid produced through the anterior pituitary, which at this time secretes thyrotropic hormone in excess. Indeed, Loeser [337] found in guinea pigs that in the first two or three weeks after removal of the ovaries there was an augmented production and secretion of thyrotropic hormone. Entirely consistent results were obtained by Franke and Ptaszek[183] in young bitches from which the ovaries were removed in acute experiments. As shown in Table 34, taken from their work, a marked increase in blood iodine was noted within the first week. Within three weeks the level was back to normal and, indeed, later dropped below the initial level. In old bitches whose ovaries, of course, were already nonfunctional, no such increase in blood iodine occurred. Franke and Ptaszek pointed out that there was a parallel rise and fall in basal metabolism simultaneously. Thus these experiments constitute a good example of the influence of the ovary upon the pituitary-thyroid axis described in Chapter VI.

Ovarian Cycle and Iodine Excretion. During menstruation the iodine excretion is increased, as shown by Cole and Curtis.[95] The iodine so lost in the urine does not conform precisely to the onset of menstruation, but in general it tends to be increased by about 15 per cent during the week of the menses. In addition, of course, there is a concomitant loss of iodine in the menstrual blood, as mentioned on the preceding page.

Pregnancy. It has long been known that pregnancy increases the demand for iodine and in consequence may produce goiter in regions where the iodine supply is low. It is not surprising, therefore, to interpret the following results of Bokelmann and Scheringer[485] as evidence of this increased demand. They found that nearly all cases of pregnancy show a detectable rise within three months, and this increases to a peak at the seventh month. From that time on, the iodine concentration in the blood is maintained at the increased level until shortly after delivery. There is considerable variation in the blood iodine during pregnancy, but many cases show blood iodine as high as 20 or 30 gamma per cent late in the course of gestation. After delivery the hyperiodemia rapidly diminishes so that many cases have normal blood iodine within two to six weeks. In female goats Leitch[314] found a hyperiodemia in heat, but normal levels during gestation until just before parturition, when hyperiodemia recurs.[313] More data are needed on this problem.

The blood from the umbilical vein also has been analyzed, and in general tends to be nearly normal, i.e., lower than the blood of the mother, in whom definite hyperiodemia occurs near the end of pregnancy.[310] Characteristic values were 10 to 19 gamma per cent. A small percentage of cases range higher, namely, according to Veil and Sturm,[560] from 19 to 55 gamma per cent.

As will be discussed in the chapter on iodine balance, there tends to be increased iodine elimination during pregnancy in spite of a poor iodine intake. This paradoxical result reminds one somewhat of the abnormally high nitrogen elimination found by Weech[569] in Chinese who were living on an inadequate protein diet. Nakamura[404, 405] found increased urinary iodine in pregnant women, as did Scheringer.[486] In general, there would seem to be increased thyroid secretion during pregnancy.[487] Thyroid hyperplasia is an accepted concomitant finding, and Schmelling[498] found that histopathological changes in the thyroid could be prevented by feeding iodine to pregnant animals. To date, however, no reliable evidence of increased thyroid hormone in the blood is available.

Thyroid and Ovary

The precise physiological mechanism by which these changes in iodine metabolism are produced is by no means clear. It is known that, in animals, injections of theelin[195] or progesterone in high dosage result in marked physiological changes in the thyroid.[268] Conversely, Scheringer[486] showed that in white rats removal of the ovary is followed by a relative increase in the thyroid iodine. Within a little over a month the concentration in the gland increases to 24 mg. per cent, in contrast to 17 mg. per cent in the control animals. Much of this apparent increase in concentration is due to functional atrophy of the tissue rather than to decrease in the total amount of iodine. This result, however, represents the end phase of a thyroid readjustment, because, as Loeser[337] has shown, immediately after removal of the ovary there is transitory histological evidence of thyroid hyperfunction. Indeed, Aron and Benoit[20] believe that castration not only increases the secretion by the pituitary of more gonadotropic hormone, but also of thyrotropic hormone. This "shotgun" reaction of the pituitary has been mentioned in Chapter VI. Furthermore, these authors gave to guinea pigs a mixture of thyrotropic hormone and estrin simultaneously. As compared with control animals receiving thyrotropic hormone alone, the effect of the thyrotropic preparation was diminished by the estrin; in the dosage used, estrin alone had no effect on the thyroid.

It has been found by Chouke, Friedman and Loeb[60] that in the female guinea pig mitotic activity in the thyroid epithelium increases during the lutein phase and reaches a minimum subsequently during the follicular phase. In some animals there is a decrease in iodine content during estrus and an increase during intervallum, as would be suspected from the parenchymatous softening of the thyroid during estrus, with engorgement of the blood vessels. Long ago, Kocher [287] found that the iodine content of the thyroid decreases during pregnancy. This presupposes, of course, that no unusual supply of iodine is available during gestation. There seems to be no question that the gland is hyperplastic during pregnancy, and that this hypertrophy or hyperplasia can be suppressed in large measure, as judged by histopathological examination, through the feeding of iodine to animals during pregnancy, as reported by Schmelling [498] (1934). The actual concentration of circulating thyroid hormone in the blood of pregnant women is still open to question, and this problem will not be settled finally until adequate methods for measuring hormonal iodine have been developed. It seems likely that the total organic iodine in the blood of mothers is increased, but it is by no means clear that this represents true circulating hormone. On the basis of histological examination, some authors claim hypothyroidism during pregnancy, whereas others report hyperthyroidism; and attempts to measure the thyroid hormone [17] in the blood by biological tests have been unsatisfactory. It remains clear, however, as shown by Marine, Cipra, and Hunt,[357] that the increased metabolism which occurs normally during pregnancy in rabbits depends upon the presence of the thyroid. It is also clear that after complete removal of the thyroid the animals do not lactate; likewise it seems probable that in the absence of the thyroid the normal preliminary hypertrophy of the mammary gland as a prelude to lactation fails to occur. Immediately after ovariectomy, Franke and Ptaszek [183] found a rise in blood iodine, together with a rise in metabolism as already noted. Beginning in the second week, the hyperiodemia decreased to the normal level in a month's time. The metabolism also fell. No such effect was found in old bitches, presumably postmenopausal. Loeser [337] showed that the effect is through the anterior pituitary, which produces increased amounts of tropic hormone, — for 18 to 19 days in guinea pigs. This temporary phenomenon, however, cannot explain protracted effects on iodine metabolism in normal animals.

As might be expected, the injection of theelin or progesterone produces changes in the thyroid.[268] Nathanson and Rawson[406] believe these to be secondary to a generalized stimulation of the pituitary in "shotgun," nonspecific fashion. Curiously enough, however, Karp and Kostkiewicz found an accumulation of colloid, distending the follicles and causing flattening of the follicular cells. Less marked effects were found by Giedosz[196] after the injection of progesterone. This seeming antagonistic effect of estrin has been described also by Starr.[518] These results are hard to reconcile with Scheringer's observation[486] that these hormones produce hyperiodemia.

Nevertheless, in the course of the sexual cycle definite changes occur in iodine metabolism. During estrus there is[404, 405] a decreased iodine content in the rat's thyroid. Chouke, Friedman, and Loeb's[86] finding that guinea pigs' thyroids show increased mitotic activity during the lutein phase, but decreased mitosis in the follicular phase harmonizes with Nakamura's iodine analyses.[404]

Reciprocal Thyro-Ovarian Relationships. In short, there are complicated interrelationships between ovarian function and the pituitary-thyroid axis. Conversely, thyroid function seems to have certain reciprocal effects upon the pituitary-ovarian axis. Without more precise methods for measuring the actual concentration of various tropic hormones and end-organ hormones in the blood, it remains difficult to assess the relative importance of the various interpolated possibilities. It probably remains possible for three general types of interrelationship to occur between the thyroid and the ovary, namely, (*a*) a peripheral sensitization of the tissues by thyroid hormone to ovarian hormone, and vice versa; (*b*) an effect of the thyroid on the pituitary-ovarian axis, or vice versa; and (*c*) an indirect effect upon other glands, e.g., the thyroid may stimulate the adrenal cortex and so influence sex activity.

Effects on the Pituitary-Ovarian and Pituitary-Thyroid Axes. At least a portion of the influence of the thyroid hormone on ovarian function is translated through the pituitary-ovarian axis; and, conversely, some of the effects of sex hormones on the thyroid, if not most of them, are translated through the pituitary-thyroid axis.

As evidence of antagonism between theelin and the pituitary-thyroid axis, Cramer and Horning[108] reported that injection of extracts containing the thyrotropic hormone prevented the characteristic effects of the prolonged administration of theelin to mice. Among these effects were (*a*) the spontaneous development of mammary carcinoma; (*b*) enlargement of the mammary gland in male mice, and (*c*) certain characteristic histological changes in the anterior pituitary, e.g., disappearance of oxyphilic granules together with vascular congestion. Conversely, Franck [182] found that after the injection of huge doses of theelin into guinea pigs, their thyroid glands were found to be "quiescent" on histological examination. Apparently there is a time element in this reaction because Amilibia, Mendizábal, and Botella-Llusiá [14] found that *at first* young male albino rats showed increased thyroid activity histologically when large doses of theelin were injected for five days. Subsequently, the thyroids reverted to a state of complete quiescence, a reversal which was thought to be due to altered pituitary activity.

Variations in iodine balance have corroborated findings that thyroidal mitotic activity was greatest during the ovarian luteal phase, and lowest during the follicular phase; and, in general, the thyroids of female animals were more active histologically than those of males. Similarly, the pigeon's thyroid appears more active (histologically) at times of sexual activity and ovulation. [458, 367]

Direct Thyro-Ovarian Interaction. There seem also to be a number of reciprocities between thyroid and ovary which may in part involve peripheral action. For example, increase in thyroid hormone within an organism tends to increase gonadal activity. Graham [204] has demonstrated an increase in the production of milk by cows after thyroid feeding, as well as increased concentration of the fat in milk. Likewise hens receiving thyroid medication lay more eggs than the controls receiving no medication.[578] Similarly, hypothyroidism [281] may be the cause of abortion, especially of "missed" variety, and Foster and Thornton [181] have used thyroid in the treatment of menstrual irregularity with considerable success in certain groups of cases. Indeed, clinicians have recognized for a long time that menorrhagia is characteristic in developing myxedema up to the point that the full-blown condition has existed for so long that body tissues in general are grossly abnormal; at this advanced stage the menses cease. Conversely, women suffering from hyperthyroidism usually report oligorrhea or amenorrhea. At the present time it is far from clear how these effects are produced. Perhaps they result from the speeding up of the metabolism of endocrine tissue, in consequence of the greater accumulation of organic iodine-containing material within the gland, as reported in Chapter VI. Perhaps, however, they are the result of indirect intervention of other glands; for example, increase in the circulating thyroid hormone leads to hypertrophy of the adrenal cortex, and this latter gland is known to act synergistically with the ovary. Similarly, Membrives [392] studied the ability of implants of the anterior pituitary into immature rats to produce vaginal canalization through ovarian stimulation, and found that this effect was augmented by feeding thyroid and diminished after excision of the thyroid gland.

As more conclusive evidence of this direct effect, the experiments of Tyndale and Levin [553] may be cited. They studied the response in the weight of ovaries (*a*) in normal rats, (*b*) in hypophysectomized rats, and (*c*) in hypophysectomized immature rats which were treated with thyroxine, when these animals were all injected with urine derived from patients at the menopause. Of course such treatment usually produces marked stimulation of ovarian follicles in consequence of the high concentration of gonadotropic hormone in such urine. Surprisingly enough, the simultaneous injection of thyroxine markedly decreased this response. These striking results, obtained in hypophysectomized animals, indicated that the inhibiting action of the thyroid was a peripheral one, not involving the pituitary gland. As further evidence of the direct effect may be cited the observation of Gessler,[194] who studied the influence of theelin on the basal metabolic rate. He showed that this ovarian hormone can decrease the basal metabolic rate both of normal guinea pigs and of hypophysectomized rats. This result he attributed to a direct antagonism between theelin and the thyroid hormone.

Interestingly enough, Starr and Patton [517] have reported remissions in human hyperthyroidism induced by the administration of extracts of pregnancy urine as well as of the pure follicular hormone. Although these clinical responses seem indubitable, they may be interpreted either (*a*) on the basis of a direct thyro-inhibitory action by theelin, or (*b*) as the result of suppression of the secretion of thyrotropic hormone by the pituitary, just as Moore and Price [399] found that an excess of gonadal hormone would suppress the production of gonadotropic hormone. Ordinarily there is little evidence that physiological concentrations of female sex hormone affect thyroid status. Thus the administration of theelin to normal dogs has no effect whatever [303] on the metabolic rate; and there is no conclusive evidence, on the other hand, that castration produces either hyperthyroidism or hypothyroidism of any considerable duration.

CHAPTER VIII

NEUROLOGICAL INFLUENCE

In nearly every case of Graves' disease, an extended discussion sooner or later raises the issue as to how much of the symptomatology is due to the nervous system and how much to strictly endocrinological chemical action. The reason for the confusion which exists about this point is fundamental, namely, that it has not yet been elucidated exactly what relation the nervous system and the thyroid hormone have to each other. The problem is at least twofold, because it involves, first, the influence of the thyroid hormone upon the action of nervous tissue, and, secondly, the action of nervous impulses of one type or another directly upon the action of the thyroid or indirectly, upon the pituitary-thyroid axis. Until recently the latter point has been particularly obscure, but at the moment it seems possible to take a more optimistic viewpoint and to believe that we are now gaining a considerable insight into the elucidation of both of these points.

Action of Thyroid Hormone on the Nervous System

There now seems to be no question that the thyroid hormone increases the activity of the nervous system (and its component tissue), and quickens reflex responses and irritability throughout the nervous system as a whole. Clinicians have long recognized this fact, noting the vivacity of the mildly hyperthyroid individual and employing mild doses of thyroid hormone to quicken the metabolism of individuals in whom sluggishness was a main feature. In the last decade strictly scientific evidence has been accumulated to justify the empirical conclusions of physicians. For example, Cohen and Gerard[90] have studied carbohydrate metabolism of brain tissue and demonstrated that surviving slices of brain tissue had an increased oxygen consumption as compared with the control tissues. A typical experiment of Cohen and Gerard may suffice to illustrate the general technique. Rats were fed 0.6 gram of desiccated thyroid each day for nearly three weeks. They were then killed, the brains removed and the cerebral tissue converted into a finely minced paste.

This "brei" was suspended in a phosphate Ringer's solution in Warburg's apparatus at 37°C., and the rate of its oxygen consumption was measured. After two hours the respiratory rate had become constant and certain substrates of carbohydrate nature were added; the respiratory rate was then measured for another hour and one-half.

These experiments showed that the rate of oxidation of hyperthyroid brain tissue was initially greater by 20 per cent than that of normal brain. Such tissue, however, became exhausted more rapidly than did the normal brain as time went on. Certain substrates could then be added to the solution and it was found that they increased the respiration of hyperthyroid brain tissue about four times as much as they increased that of normal brain; among such substances were succinate, fructose, lactate and glucose. These experiments suggested that in the hyperthyroid nervous system certain enzyme systems were activated or "enhanced." Thus, one has a chemical basis for presuming increased activity of the nervous system in hyperthyroidism. This increased irritability is apparently related to enzymes which prepare substrates of carbohydrate nature for oxidation.

With the advent of electro-encephalography, furthermore, it has been possible to verify the fact that the brain as a whole *in vivo* has an inherently greater activity in the hyperthyroid than in the normal individual; and, indeed, a lower inherent activity in the hypothyroid state. For example, Ross and Schwab [463] have studied the cortical alpha-rhythm in thyroid disorders and found that, whereas the normal alpha-rhythm is 10 per second, that in Graves' disease may be as high as 14, and that in myxedema as low as 6 per second. Similarly, the normal reaction time is about 0.23 second, whereas in hyperthyroidism it may be as low as 0.2 second and in myxedema as high as 0.5 second.

A generation ago Oswald [422] called attention to this so-called "exquisite manner" of increasing nerve tone possessed by thyroid hormone. This increased tone is evident not only in the brain and other parts of the central nervous system, but is also apparent in the vegetative nerves, as evidenced by increased vasomotor action and peristalsis of viscera. Of course in hypothyroidism the reverse is true.

A great deal more evidence could be added to the experiment just cited, showing that in hyperthyroidism the nervous system is hyperactive and, in hypothyroidism, sluggish. These observations, however, do not directly concern iodine metabolism, because apparently they simply reflect the presence of increased thyroid hormone. There is some pharmacological evidence that high concentrations of iodide do suppress nerve irritability, much as bromide does. Indeed, this phenomenon has been utilized in epilepsy and in related conditions. It is much less effective than bromide, however, and certainly belongs in the realm of pharmacology rather than physiology, normal or pathological. The evidence just cited is given here merely to show that a good deal of confusion may arise from the finding of a hyperactive nervous system in association with hyperthyroidism. The observer is never quite sure whether the nervous activity is cause or effect. Does the hyperactive nervous system stimulate the thyroid to increased activity? This brings us to our second and more cogent problem.

Action of the Nervous System upon the Thyroid

Before proceeding to consider whether or not, or to what extent, the nervous system influences the thyroid, it must be made clear that the problem is not merely a neurological one. The more important problem perhaps is to what extent the nervous system may control the pituitary-thyroid axis.[70] The problem becomes multiple, because in a given case of hyperthyroidism one might have at least two possibilities, namely, increased stimulation of the thyroid directly by increased activity of sympathetic nerves; or, secondly, increased stimulation of the pituitary by the nervous system with resulting increased secretion of thyrotropic hormone, which in turn would lead to increased thyroidal activity. The multiplicity of pathways, neural and humoral, by which the thyroid may be stimulated has been appreciated in recent years and led to the widespread use of the term "neurohumoral" mechanisms. Fig. 22 illustrates such relationships. Thus the problem of thyroid stimulation becomes quantitative rather than qualitative; that is, in analyzing the neurohumoral mechanism which obviously controls the thyroid, the essential point is to distinguish the relative influence which each of these two members plays in the final result, and to decide to what extent they act independently and to what extent as part of a coordinated system.

As more data accumulate on this question, the importance of the thyrotropic hormone as the final common path by which intrinsically nervous activity is impressed upon the thyroid becomes more and more impressive. Indeed, granted that the hypothalamus is the seat of coordination of emotional tone, it is becoming apparent that both the sympathetic nervous system and the endocrine system are uti-

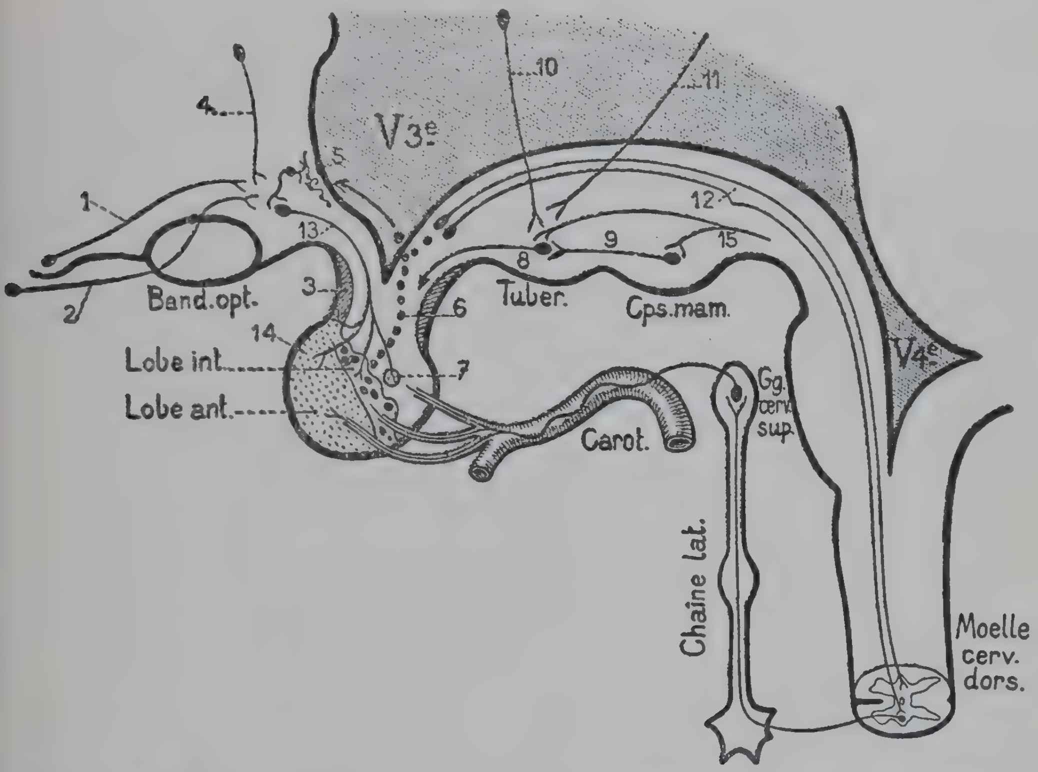


FIG. 22. The innervation of the pituitary body.

Band. opt.: optic tract; *Carot.*: carotid artery; *Chaîne lat.*: cervical sympathetic; *Cps. mam.*: mammillary body; *Gg. cerv. sup.*: superior cervical ganglion; *Lobe ant.*: pars glandularis; *Lobe int.*: pars intermedia; *Moelle cerv.-dors.*: cervico-thoracic portion of spinal cord; *V3*^{e}: third ventricle; *V4*^{e}: fourth ventricle; *Tuber*: tuber cinereum.

(*1*) Amygdalo-tangential* tract (olfactory-pituitary reflexes). (*2*) Retino-tangential* tract (optico-pituitary reflexes). (*3*) Pars tuberalis. (*4*) Pathway by which pituitary can be influenced by corpus striatum and globus pallidus. (*5*) Subependymal network of sensory nerves. (*6*) "Neurocrinie hypophyso-hypothalamique." (*7*) Islet of glandular cells in pars neuralis. (*8*) Tubero-hypophysial tract. (*9*) Mammillo-hypothalamic tract. (*10*) Thalamo-hypothalamic pathways. (*11*) Cortico-hypothalamic pathways. (*12*) Decussation of descending hypothalamic pathways. (*13*) Hypothalamico-hypophysial tract. (*14*) Zone of transition. (*15*) Central sensory pathways.

*Tangential or supraoptic nucleus.

After: G. Roussy and M. Mosinger, Presse méd. *44*: 1521. 1936.

Key as adapted by H. B. van Dyke, The Physiology and Pharmacology of the Pituitary Body, vol. 2, p. 7, Chicago, Univ. of Chicago Press, 1939.

WARNING. As pointed out in the text, the direct hormonal influence of hypothalamic centers, indicated by *6*, has been excluded by the work of Wislocki. Furthermore, the true anatomical relationships are somewhat distorted in this diagram, as if the horizontal axis had been stretched. Thus the bulge at the point marked *8* should lie almost directly above the posterior lobe of the hypophysis, and might better be termed the "posterior infundibular eminence." See note under Fig. 23.

lized by this central reflex organ to control bodily processes. The pituitary is situated at the base of this organ and nerve fibers run to it through the stalk from the region of the supra-optic nuclei. This pathway leading to the posterior lobe, as shown in Fig. 23, is concerned with water balance. Heat elimination is controlled from sites lying between the optic chiasm and the anterior commissure, i.e., in the supra-optic (SO) and pre-optic regions. J. C. White has discussed in detail the functional aspects of these hypothalamic centers in "The Autonomic Nervous System," revised second edition, Macmillan, 1940–41. Of special interest, however, is the region

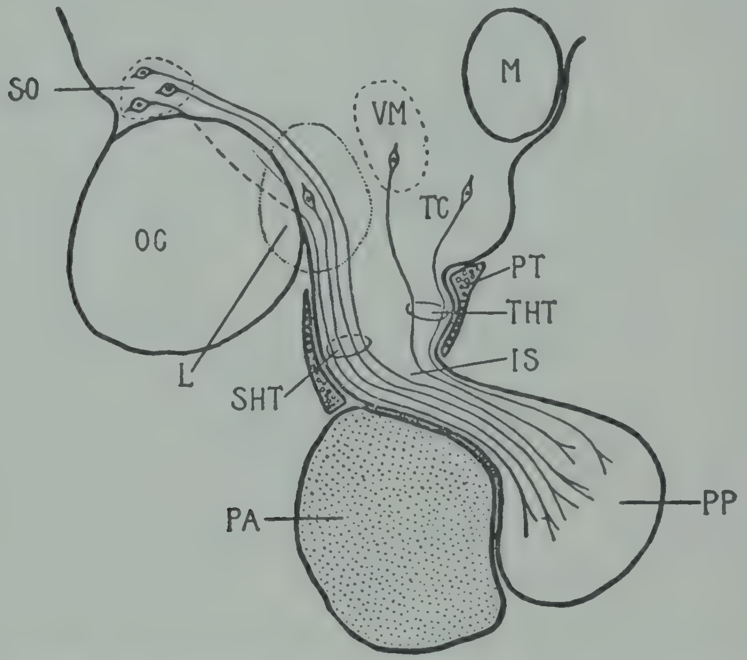


FIG. 23. Probable neural interrelationships of the hypothalamus and pituitary of the monkey, showing the location of lesions (circumscribed region *L*) which produce diabetes insipidus.

IS: stalk; *M*: mammillary body; *OC*: optic chiasm; *PA*: pars glandularis; *PP*: pars neuralis; *PT*: pars tuberalis; *SHT*: supraoptico-hypophysial tract; *SO*: supraoptic nucleus; *VM*: ventromedial component of supraoptic nucleus; *TC*: tuber cinereum; *THT*: tubero-hypophysial tract.

From: W. R. Ingram, C. Fisher and S. W. Ranson, Arch. Int. Med. *57*: 1076. 1936. Key as adapted by H. B. van Dyke, The Physiology and Pharmacology of the Pituitary Body, vol. 2, p. 8, Chicago, Univ. of Chicago Press, 1939.

NOTE: For a precise description and an exhaustive discussion of the diencephalon, together with revised terminology pertaining thereto, see "The Hypothalamus and Central Levels of Autonomic Function," Research Publications, Association for Nervous and Mental Disease, volume XX, Williams and Wilkins Company, Baltimore, 1940. This diagram fails to show nerve fibers leading to the anterior lobe. As discussed in the text, this question is still *sub indice* but the investigations of Brooks and Gersh, and of Hair indicate that such tracts probably exist.

above the mammillary body (M) on either side, about the paraventricular nucleus.

This comprises the region which is concerned with the regulation of heat production, as shown in Fig. 23. In a syndrome like exophthalmic goiter, the precise etiology of which is unknown at present, one finds many manifestations of both neural and hormonal activity. In addition, one finds increased irritability of the nervous system due to the intense, exquisite stimulation of nerve tissue, to use Oswald's phrase. All three of these factors must be considered in evaluating the total situation in Graves' disease, and in trying to discern cause and effect. There is good evidence that both nerves and hormones may thus influence iodine metabolism, but the precise pathways and sequence of events still remain a puzzle.

Thyroid Nerves. There is much work in the literature to indicate that the thyroid gland receives fibers both from the sympathetic and the parasympathetic divisions of the autonomic nervous system. The first type of fiber is derived from the cervical ganglia and the latter from the vagus nerve. These fibers run along the branches of the laryngeal nerve and enter the parenchyma of the gland with the blood vessels. In recent years Nonidez [412] has studied the thyroid nerves in the dog in great detail. He has followed both sympathetic fibers from the superior cervical ganglion and parasympathetic fibers from the ganglion nodosum of the vagus into the thyroid nerve, which is a branch of the superior laryngeal nerve. Ramifications of the last named enter the gland along with the superior arteries. In addition, there are fibers from the carotid plexus which penetrate into the gland within the coats of the arteries. There are also large bundles of fibers which are distributed in the parenchyma apart from blood vessels. Within the gland substance are collections of ganglion cells resembling the parasympathetic ganglia found in the trachea and larynx. In addition, there are occasional ganglion cells scattered throughout the gland.

Beside the nonmyelinated end-fibers of the two branches of the autonomic system, there are also myelinated fibers, some of which are probably sensory fibers conducting afferent impulses into the sympathetic cerebrospinal ganglia. There are also myelinated fibers which presumably connect with the thyroid ganglion cells as preganglionic fibers of the parasympathetic system from the medulla. Although these fibers form complicated plexuses within the gland, there is no definite evidence [413] that they connect directly with follicular cells lining the thyroid follicle. To be sure, there are some free interfollicular endings, but these are probably sensory, according to Nonidez.[412] Of course the thyroid has a rich vascular supply and about these vascular ramifications nerve endings, both sensory and motor, are found in great abundance. Modell [396] has described arteriovenous communications in this vascular maze, and these also are well supplied with nerve endings. Thus, the anatomical evidence to date indicates control of vascularity rather than direct parenchymal control by the autonomic nervous system.

The influence of these nerves supplying the thyroid has long remained obscure, and contradictory answers have been returned to most of the important aspects of this problem. Much of the confusion has been centered about this question: does the thyrotropic hormone stimulate the thyroid in the absence of sympathetic nerve supply? In 1930 Dobrzaniecki and Aron [122] said no; but later Krayer [299] said yes *in vivo* and Eitel, Krebs, and Loeser [134] said yes *in vitro*. Likewise, Weil (1936) found that autogenous thyroid transplants respond to injections of thyrotropic hormone. Presumably, thyrotropic action does not require intact nerve pathways.

In addition Van Dyke [557] found no appreciable changes in thyroidal iodine, or histological picture after unilateral stimulation of the sympathetic nerve supply to one side of the gland. Likewise, Hektoen, Carlson, and Schulhof [235] found no augmented thyroglobulin in the thyroid vein or lymph on the stimulated side.

In 1915 Cannon and his coworkers [72] performed the Langley operation, anastomosing the phrenic nerve with the distal end of the cervical sympathetic trunk. They succeeded in producing in four cats a periodic dilatation of the pupil of the eye synchronous with respiration. Simultaneously they found increased oxygen consumption by these animals, associated with generalized nervousness, increased loss of weight and changes in the thyroid consistent with overactivity. In recent years, however, Friedgood and Cannon [188] have concluded that the thyroid stimulation found in these early experiments of Cannon, Binger, and Fitz [73] actually took place by way of the pituitary.

More recent studies by Uotila [555] indicate that an increase or decrease in an organism's thyroxine level acts on the anterior pituitary without any mediation, or modifying action of the pituitary stalk. This conclusion seems warranted by a number of facts, among which are the following. When thyroxine is given to male rats in daily doses of 5 mg. per kilogram, marked atrophy of the thyroid ensues within 10 days whether the pituitary stalk be intact or severed. Furthermore, even after destruction of the pituitary stalk, residual thyroid tissue is still capable of compensatory hyperplasia. Of course, this basic thyrotropic secretory rhythm possibly is subject to regulation by nerve impulses arriving from the hypothalamus through the pituitary stalk, or, to a lesser degree, by way of the cervical sympathetics.

Revived interest in the autonomic control of thyroid function has received further impetus from recent observations of Friedgood and Cannon, who in two of 28 cats reproduced the syndrome of Cannon, Binger, and Fitz by unilateral phrenico-sympathetic anastomosis. In one of these animals, removal of the ipsilateral thyroid lobe was followed by a moderate but progressive decrease in the basal metabolic rate. Indeed, after resection of the anastomosis, the basal metabolic rate became normal within 10 days. Many details of the relationship between thyroid and the autonomic system remain to be discovered. For example, why does unilateral exophthalmos occur, together with the increased metabolism in these animals? Another finding which also raises many questions is that both adrenine and pilocarpine augment the thyroid-stimulating activity of an anterior pituitary thyrotropic extract. In short, it is already clear that nervous activity may regulate thyroid activity, but the mechanisms are obscure because of anatomical complexity and because increased thyroidal activity reacts upon the nervous system.

Furthermore, Smith and Moloy[513] have searched for changes in the size, number, and shape of the mitochondria of the thyroid cells after stimulation electrically of the thyroid nerves. Although they regard this test as a good indication of functional activity, they failed to find any such evidence after the nerve stimulation. There is no question, however, that the thyroid nerves do bring about marked vasomotor changes within the gland, affecting its volume and its circulation on the basis of blood flow. Thus Mason, Markowitz, and Mann[368] measured the volume of the intact thyroid of dogs in a plethysmograph; and demonstrated a change of volume as the result of nerve stimulation and also after administering certain drugs, like ephedrine, pilocarpine and acetylcholine. Thus sympathetic stimulation caused definite vasoconstriction. Similar results were described by Vogt.[562]

Direct Control of the Thyroid

In a brilliant piece of research recently executed by Uotila,[555] the reaction of the thyroid *in vivo* to cold was used as the means of tracing thyrotropic and sympathetic stimulation. In order to measure the activity of the thyroid directly, the average cell height of the thyroid follicular cells was measured microscopically with an ocular micrometer. The sections were fixed, of course, in the usual histological manner so that the data which are to be summarized must be considered relative, although the figures will be given in terms of micra. It has been known for a long time that after hypophysectomy the height of the follicular cells drops as the gland assumes the so-called "resting" state. Uotila's [555] figures showed a drop from about 3.5 micra to a little over 2 micra in cell height within two weeks after hypophysectomy, as shown in the accompanying Fig. 24. This same phenomenon occurred whether the animals were kept warm or cold. As shown in the second half of Fig. 25, if control animals were kept at icebox temperature the cell height increased within a few days definitely above 4 micra and sometimes as high as 7 micra. It is known that similar effects can be obtained by injecting preparations of thyrotropic extract made from the anterior pituitary. Taken together with other indications of secretory activity, e.g., secretion vacuoles, the loss of colloid and changes in staining, such figures afford a good index of thyrotropic effects. They likewise would serve as a good index of activation of the thyroid by nervous activity in the present instance. Thus Uotila found, as did Wolf and Greep,[580] that the activation of the thyroid by cold depends upon the presence of the hypophysis, or of its thyrotropic hormone.

This investigator also showed that extirpation of various parts of the cervical sympathetic system failed to produce much direct systemic thyroid effect or any clear-cut alteration of the histological reaction of the thyroid. Clearly, then, the evidence indicates that the thyroid, under this common physiological circumstance, is activated not by cervical sympathetic nerves but by the tropic hormone from the anterior pituitary, and this chemical activation is the principal mode of control. Unilateral cervical sympathectomy in the rat did not affect the ipsilateral thyroid lobe, although bilateral cervical sympathectomy did cause a temporary and very mild hypoactivity of the gland. Similarly, the cold reaction was modified temporarily. Thus transient effects of cervical sympathectomy may occur, but they appear to be compensated for by other pathways. The sympathetic nervous system, in so far as its cervical portion is concerned, does not appear necessary for the continued tonic effect required to maintain normal thyroid function.

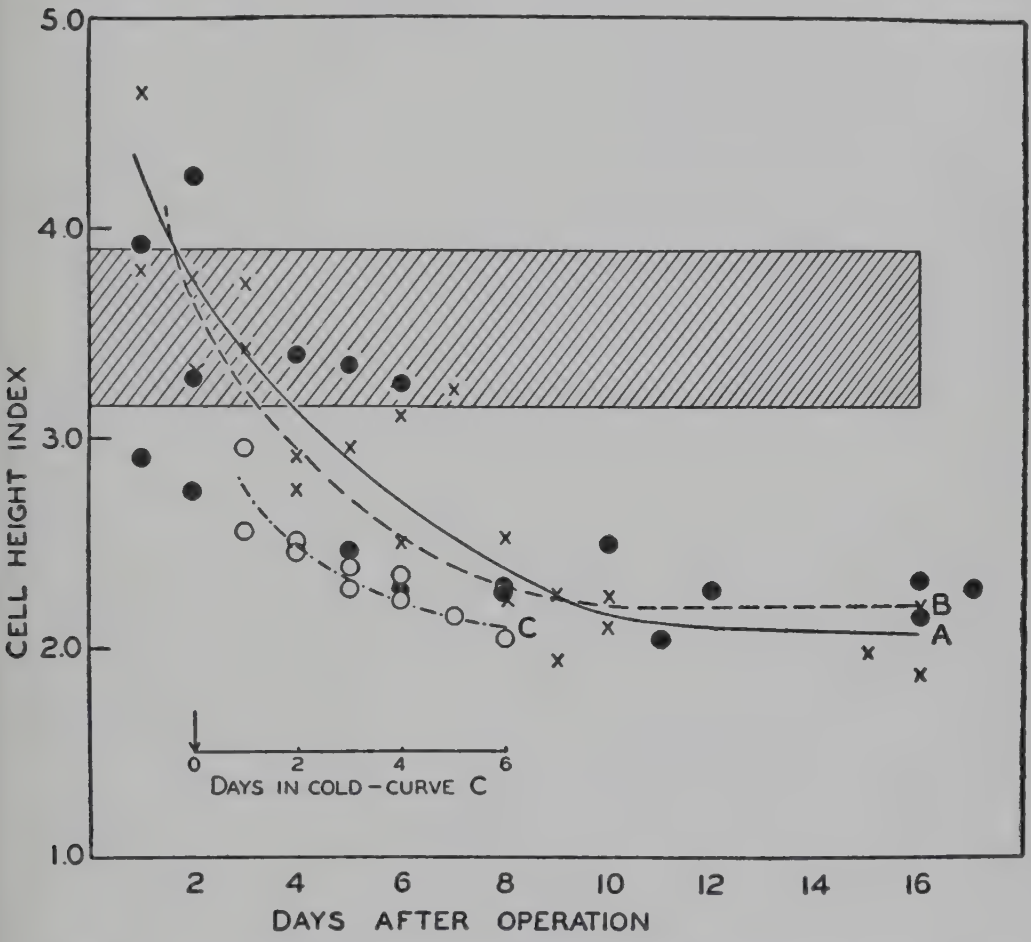


Fig. 24. The influence of hypophysectomy on thyroid activity, as indicated by the height of the thyroid acinar epithelium. Curve A (crosses) shows the effect of hypophysectomy. Curve B (black dots) shows the result of hypophysectomy combined with bilateral cervical sympathectomy. Curve C (circles) shows the effect of cold upon hypophysectomized animals. The normal variation of cell height in control animals is indicated by the shaded area.

From: U. U. Uotila, Endocrinology, *25*: 65. 1939.

INDIRECT CONTROL OF THE THYROID

Uotila [555] went further, however, and severed the pituitary stalk in certain rats. At ordinary room temperature these animals be-

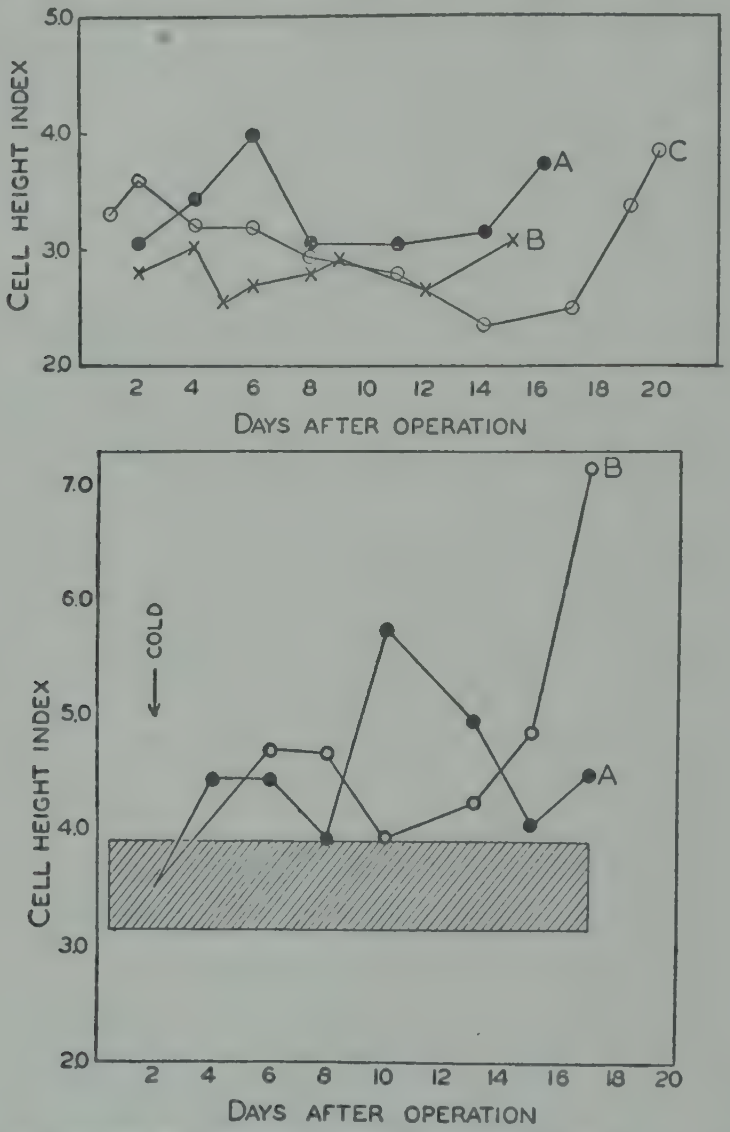


FIG. 25. The influence of sympathectomy upon the activity of the thyroid, as indicated by the height of the acinar epithelium. For contrast with the preceding figure.

In the upper graph, Curve A (black dots) shows merely the result of a blank operation; Curve B (crosses) shows the effect of left cervical sympathectomy; and Curve C (circles) the result of bilateral cervical sympathectomy. Obviously, these effects are all slight, at best.

In the lower graph, the reaction to cold is recorded after bilateral cervical sympathectomy. Curve A (black dots) shows merely the result of cold after a blank operation; Curve B (circles) shows the stimulating effect of cold after bilateral cervical sympathectomy. The shaded area indicates natural variation in the control animals, which were not operated upon.

From: U. U. Uotila, Endocrinology, *25*: 67. 1939.

haved much like the normal controls and their thyroids showed approximately the same cell height. There was no regression in the gonads or in the thyroid. The pituitary itself showed little change except perhaps a slight diminution in the eosinophilic elements. When both groups of animals were kept in the cold, at 4 to 5°C., however, within four or five days marked hypertrophy could be noted in the follicular cells in the thyroids of the normal animals. By contrast, the animals whose pituitary stalk had been cut failed to show any activation of the thyroid. Actually, the animals' body temperature fell and because of this many of them died soon. Thus, continuity of the pituitary stalk seemed to be essential for the reaction of these animals against cold, even though this reaction has been demonstrated as due to thyrotropic hormone. In short, here was concrete evidence of a collaboration, or synergism, between the autonomic centers in the hypothalamus and the pituitary-thyrotropic axis.

This work of Uotila's is particularly important because it offers an opportunity of reconciling many apparently contradictory results as to the relative effect of the tropic hormone, on the one hand, or of the sympathetic nervous system, on the other, in influencing thyroid activity.

Neurohumoral Coordination. Just as a generation ago Sir Charles Sherrington [507] compiled evidence to illustrate the integrative action of the nervous system, so in the past generation evidence has been accumulating which now may be synthesized into a concept of the integrated neuro-endocrine system. Beginning with the demonstration in 1912 by T. R. Elliott that stimulation of the splanchnic nerves released the hormone, adrenine, evidence has been accumulating that the stimulation of nerves releases chemical substances which have a specific mission. Thus Cannon and Rosenblutt [74] have shown that sympathin is liberated on sympathetic stimulation, just as Dale [117] and Loewi [340] have shown that acetylcholine is liberated by parasympathetic stimulation. It requires only a slight effort of the imagination to see by analogy that it is possible for nerve fibers ending in the pituitary to liberate a hormone which exerts its effect specifically at a distance.[118] Various papers by Fisher, Hare, Hair, Ingram, Magoun, and Ranson have stimulated thought along such lines. Within a few years Rasmussen [449] has shown that there are approximately 50,000 nerves in the pituitary stalk of man. Moreover Ranson and his associates[351] have correlated this finding of nerve fibers by enumerating cells in the supra-optic nuclei after section of the infundibular stem and adjacent loci. Whereas such counts show about 34,000 cells in the monkey's supra-optic nucleus, unilaterally, this number decreases to less than 10,000 after section of the stalk at the sella turcica. Indeed, after section higher up, through the median eminence or the anterior half thereof, the count is only some six or seven thousand. By contrast, a lesion through the posterior half of the median eminence reduces the count only to 27,000. In short, this retrograde degeneration indicates indubitably that there are well-defined nerve tracts running between the hypothalamic region and the hypophysis by way of the pituitary stalk. Some of these terminate in the pars tuberalis and are distributed over the median eminence. Moreover, Gersh[193] has found numerous terminals surrounding the posterior lobe in a complex network.

In the Dunham Lectures for 1940 at the Harvard Medical School, Ranson reviewed the available evidence bearing upon the role of the hypothalamus as a regulator of mammalian metabolism, development, and behavior. There is no question that this relatively small portion of the nervous system is intimately concerned with emotional and other reactions involving the autonomic system. It is concerned also with active wakefulness, lethargy, and sleep; with temperature regulation; with sexual development and sexual performance; with obesity; and with water balance, or, per contra, with diabetes insipidus.

Already it is clear that a close functional relationship exists between the diencephalon and the posterior lobe of the hypophysis. The experiments of Ranson, just cited, together with the work of Gersh and of Brooks,[66] show that this connection is maintained through the prominent supraoptico-hypophysial tract which runs in the pituitary stalk. After section of this tract, the neural hypophysis below the lesion atrophies and fails to elaborate the antidiuretic hormone. Diabetes insipidus ensues, provided the anterior hypophysial lobe remains intact.

Similar conclusions were reached by Dandy, working with human patients. During the remarkable diureses exhibited by such individuals excessive amounts of iodine may be lost in the urine. This negative balance usually is promptly restored if the diabetes insipidus is controlled adequately (Blotner and Perkin, 1940).

Unfortunately, at the present time (1940) there exists no such clear-cut evidence of a functional interrelationship between the hypothalamus and the *anterior* lobe of the pituitary, in Ranson's opinion. Such an arrangement, however, has by no means been excluded and, indeed, deserves further intensive research. There is a tubero-hypophysial tract running from the tuber cinereum through the stalk to the anterior lobe; [215] and nerve fibers possibly may also run from vascular arterio-plexuses via the circle of Willis. Thus there exists the possibility of multiple innervation of the anterior pituitary, a fact which renders the problem rather complicated technically. In addition, a humoral connection exists by way of the circulation from the anterior lobe veins to the cavernous sinus; although the once popular "portal circulation" of the pituitary has been excluded by Wislocki "as playing any significant role in conveying blood from the pituitary complex to the hypothalamic centers" (Anatomical Record *72*: 149. 1938).

Although the existence of nerve fibers in the anterior lobe seems clear,[215] the experimental evidence to date indicates that functionally the hypothalamus exerts only a vague, long-term supervision over the secretory activity of the anterior pituitary. The search for acute metabolic changes following section of the stalk, for example, has not disclosed a subsequent hypoglycemia or an increased sensitivity to insulin. Nevertheless, there is an ever-increasing mass of clinical evidence in the general field of "psychosomatic medicine" which points to nervous regulation of endocrine activity. Furthermore, these observations harmonize well with the experiments on temperature regulation by Uotila, cited in this chapter.

Further careful investigation is needed before one can identify confidently definite tracts of fibers leading from the hypothalamus to the anterior hypophysial lobe. The extant anatomical evidence of nervous control of the anterior lobe cells is imperfect, at best, although interesting pioneer studies in this direction have been made by several investigators. Among these may be mentioned the work of Hair,[215] of Brooks,[65, 66] and of Rasmussen.[449]

It is impossible to survey here in any detail the work of Brooks and others, indicating the relations of the diencephalon to various physiological processes. In summary, it seems clear that the anterior part of the hypothalamus and the posterior lobe of the pituitary are concerned with water balance, because lesions either in this lobe or higher up in the path to the supra-optic nucleus may lead to diabetes insipidus. Conversely, Gersh[193] has shown that in rats starved of water an entire neurological complex in the region of the pars tuberalis may show hypertrophy, thus indicating the nerve system which has to do with diabetes insipidus when injured. More posterior in the hypothalamus is the part of the nervous system regulating heat production.* Thus one might predict experimental loss of heat regulation, as demonstrated by Uotila in the experiments just discussed. Under conditions of excessive heat, presumably both of these neurohumoral systems would be involved, each calling into play its own end-organs, the one favoring water elimination, the other conserving heat production. Presumably, also, both of these mechanisms might be involved in an emotional or subconscious adaptation reaction. For example, clinicians have long recognized that the onset of Graves' disease might follow a severe nervous shock or fright.

Because many investigators have suspected that Graves' disease is primarily a disturbance of the diencephalon, it has been suggested frequently that sedatives in rather large doses might quell excessive nervous activity in the hypothalamic region[497] and suppress the real cause of the hyperthyroidism. To this end Gutzeit and Parade[213] treated seven patients with basal metabolic rates ranging from plus 43 to plus 100 per cent for several days (three to eight), with "prominal" in doses of 0.2 gram three times a day. At the same time they followed the "anorganische" and "organische" fractions in the blood iodine, together with their "iodine quotient." The results were rather disappointing. In reviewing ten such observations they summarized the effect of treatment on basal metabolic rate, total blood iodine and "iodine quotient," as follows. The basal metabolic rate fell somewhat in four of ten observations. The total blood iodine fell somewhat in only two cases and rose slightly in two others. The iodine quotient showed slight rise in two cases and a very slight fall in two other cases. In short, the laboratory data agreed with the clinical impression that this therapy offered no great prospect of practical application.

In this connection the relation of the sympathetic nervous system to gonadotropic function is interesting because it presents an analogy for understanding thyrotropic function. For example, the experiments of Friedgood [187] indicate that sympathectomy can diminish the frequency of pseudopregnancy after stimulation of the cervix uteri, electrically or mechanically. Furthermore, the amenorrhea of young women immigrating to this country has been classical in the last generation. Every poulterer knows, also, that fowls or pigeons will not lay eggs when moved into unfamiliar surroundings. In short, complex neurological activity in the cerebral cortex may influence the hypothalamic region and thus set off discharges simultaneously, (*a*) through the sympathetic nervous system directly to viscera, and also (*b*) through the anterior pituitary, which translates them into tropic hormones which, in turn, influence the activity of endocrine glands, thus moderating their chemical control of the tissues at large. Obviously the situation is so complex that it is often difficult to decide how much of a given net effect is to be attributed directly to nerve tissues, or indirectly to chemical hormonal influence, because in general these activities should tend to operate toward the same end under normal conditions.[556]

---

* The regulation of heat *elimination* by the pre-optic or supra-optic nucleus is dismissed here because the pituitary seems not to be involved.

This work on the action of the nervous system does not vitiate the obvious point that the thyrotropic hormone can stimulate thyroid tissue quite apart from nerve action. Thus, Marine and Rosen [363] have studied the effect of the thyrotropic hormone upon transplanted thyroid and upon grafts from glands of other animals. They found that such transplanted tissue responded with as much hyperplasia under the influence of the thyrotropic hormone as did the naturally located gland. One must rather think of each of these mechanisms as modifying the effect of the other. Indeed, Pieper [438] found that when small doses of thyrotropic hormone were given to animals, the denervated thyroid in consequence showed less hyperplasia than did an intact gland; on the other hand, with large doses of thyrotropic hormone, denervation appeared to have no influence.

Role of Iodine in Neurohumoral Integration. The place of iodine in this complex situation may be divided arbitrarily into two types. In the first place, when thyroid activity is enhanced and its secretion increased, the general body economy will be speeded up and there will be a tendency to utilize more thyroid hormone and more iodine. As the concentration of thyroid hormone in the circulating fluid increases, however, such increase will tend to react upon the pituitary and diminish the output of the tropic hormone. In this way the pituitary-thyroid axis will respond as described in Chapter VI. It is by no means clear exactly how this countermechanism is set into operation. Perhaps the increase in thyroid hormone is recorded initially in the interbrain. Further crucial experiments are needed in animals whose pituitary stalk has been interrupted. In general, of course, the total effect of any disturbance of normal balance will be checked by homeostatic mechanisms. Thus, Houssay, Mazzocco, and Biasotti[256] (1931) damaged the tuber cinereum of dogs and found that in the first month thereafter the blood iodine rose to nearly 40 gamma per cent; but it decreased a few weeks later to 20 gamma per cent. In similar fashion Schittenhelm and Eisler[495] attempted to study the effect upon iodine metabolism of removal of different parts of the brain. These authors thought they found changes in iodine metabolism, but repetition of this general type of experimentation by Sturm and Schneeberg[530] indicated that no great changes in iodine utilization followed. Bilateral destruction of the tuber cinereum was found to produce only a slight increase in urinary iodine elimination, and further lesions of the hypothalamus, the thalamus and even of the putamen and pallidum, as well as the posterior pituitary and its associated stalk, produced no very striking effects. Further work is necessary before the contradictory literature on this subject can be evaluated properly. Possibly some experiments cause hypothalamic irritation, whereas others remove important nerve centers, thus yielding opposing results.

If the foregoing considerations on the role of diencephalon in iodine metabolism be correct, it is puzzling that one can not show a change in iodine metabolism following the administration of drugs which depress this tissue. Thus far such narcotics, e.g., "luminal" and "prominal," have appeared not to influence significantly the iodine metabolism of normal individuals or animals. Occasionally, in hyperthyroidism there may be a distinct fall in the total metabolism and in the blood iodine, according to Fenz and Uiberrak.[173]

There are good grounds, however, for believing that iodine plays an intimate role in the relationship of pituitary to hypothalamus. This evidence revolves about the fact that the iodine concentration of the pituitary is high as compared with that in other tissues, and, likewise, certain portions of the diencephalon probably have very high iodine contents. At this time it is impossible to be dogmatic about these statements, because several investigations of the past decade disagree as to the facts. There is little question, however, that the pituitary selectively concentrates thyroxine which has been injected into the blood stream. Indeed, concentrations as high as 2400 gamma per cent of iodine have been reported by Sturm and Schneeberg[530] (see Löhr and Wilmanns[343]) in dogs treated for four days with "thyroid" in poisonous doses. Under these circumstances, the concentration in the tuber cinereum reached about 564 gamma per cent.

As regards the iodine in the tuber cinereum there is much dispute. After injections of anterior pituitary extracts into dogs, Pighini[439] found that the hypothalamic iodine rose from 666 to 2690 gamma per cent. He believes, therefore, that diencephalon has a peculiar affinity for some type of iodine compound. Likewise, Schittenhelm and Eisler[492] found concentrations ranging from 345 to 708 gamma per cent in the thalamus and tuber cinereum, as contrasted with values of 17 for the pallidum and 72 for the cerebellum. Furthermore, they have reported that, within two days after removal of the thyroid, no such phenomenal concentrations were found in the hypothalamus. Sturm,[528] who failed to find this effect, *did* note that after hypophysectomy in dogs the high normal concentration in the tuber cinereum dropped to the very low level of 10 gamma per cent. Thus, there seems to be a distinctly high iodine concentration in the tuber, but the basis for it is not decided. Conservative values are given in Table 35.

According to Schittenhelm and his collaborators the tuber shows much the same affinity for thyroxine as does the pituitary. Although neither inorganic iodide nor diiodotyrosine is selected by this section of the brain, concentrations over 3000 gamma per cent were found in rabbits four hours after the injection of thyroxine (1 mg. per kilogram of body weight). Curiously enough, these phenomenal increases were not attained in athyreotic organisms.

It would be pleasant to accept the hypothesis of Schittenhelm and Eisler that the hypothalamus is a central controlling station of iodine metabolism and the thyroid merely a sub-station where hormone is manufactured, stored and liberated on demand. At best, however, the evidence can be considered merely suggestive at present, pending further work with improved techniques.

TABLE 35

IODINE CONTENT OF HUMAN BRAIN

PALLIDUM		CEREBELLUM		MEDULLA OBLONGATA		THALAMUS		TUBER CINEREUM	
Wt. of brain analyzed in g.	Iodine content in γ %	Wt. of brain analyzed in g.	Iodine content in γ %	Wt. of brain analyzed in g.	Iodine content in γ %	Wt. of brain analyzed in g.	Iodine content in γ %	Wt. of brain analyzed in g.	Iodine content in γ %
14.4	12.43	15.59	5.77	7.73	10.09	4.0	18.25	2.75	32.73
14.4	12.43	18.70	5.40	9.27	9.71	4.87	18.48	2.8	32.14
4.32	12.96	15.12	8.99	8.88	7.66	11.87	11.36	2.53	25.69
18.92	12.53	20.16	8.98	8.88	8.78	5.98	11.37	5.00	25.60
18.92	13.16	13.20	7.80	6.40	6.72	11.09	27.95	4.25	27.76
15.29	17.13	13.20	6.82	6.40	8.28	11.09	28.49	...	....
15.29	16.41	17.60	6.70	9.60	6.77	13.31	22.69	...	....
15.29	14.52	13.20	6.51	...	...	11.09	28.76	. .	....
Average iodine content in γ %	13.95	....	6.36	...	8.29	....	20.92	...	28.78

A. Sturm and R. Schneeberg, Ztschr. f. d. ges. exper. Med. *86*: 668. 1933.

PERIPHERAL NERVOUS EFFECTS

It is also possible that nervous impulses at the periphery may alter iodine metabolism in the tissues. In fact, Veil and Sturm [560] found that the blood iodine was altered by substances which paralyzed or excited the autonomic nervous system. Thus they showed that after parasympathetic paralysis (with atropine) the blood iodine curve resembles that obtained by stimulation of the sympathetic system with adrenine. A rapid increase in blood iodine occurs after subcutaneous injection of atropine, rising to a peak in about half an hour and later falling rapidly. Pilocarpine, choline, and digitalis give a converse effect. Pilocarpine depresses the blood iodine for two hours. Choline produces a slight transitory drop, followed by a marked rise for two hours — chiefly of inorganic iodide. After repeated injections of digitalis, definite hypoiodemia occurs; as the digitalis is slowly being metabolized marked and prolonged hyperiodemia persists. Eventually the blood returns to normal. Of course, the thyroid and ovary may be involved in such peripheral responses.

When Schittenhelm and Eisler cut the spinal cord at various levels they obtained a prompt and definite decrease in the blood iodine. The concentration fell as low as 2 gamma per cent, but rose to normal within six hours. If hypothalamus were first removed, the fall in blood iodine was not marked. Likewise, the action of adrenaline on the blood iodine level stops after removal of diencephalon and cutting of the spinal cord between the fourth cervical and the third thoracic roots. Section of the lower part of the cord has no influence on the adrenine effect. This evidence suggests rather definitely that spinal sympathetic nerves are involved in the regulation of iodine metabolism.

Likewise, the adrenal medulla plays a small role in iodine metabolism.[488] After a subcutaneous dose of adrenine (0.8 mg.) in man, hyperiodemia to the extent of from 3 to 6 gamma per cent appears in 45 minutes, to reach a peak in one and one-half hours. After three hours the blood iodine returns to a subnormal level.[560] More marked effects were noted by Mislowitzer, Nissen, and Stanoyévitch (1934), after intravenous injection (up to 0.1 mg.). The iodine peak appeared almost immediately, amounting to a rise of from 13 to 30 gamma per cent, and dropped slowly to normal in 24 hours. In vagotonia Veil and Sturm[560] found an inverse effect at the start.

The source of the extra iodine in the blood is not clear, but it seems to be largely "anorganische." Sturm[527] found that after adrenine treatment the isolated thyroid liberates iodine if its perivascular sympathetic nerve plexus is maintained. On the other hand, Schittenhelm and Eisler[494] found that thyroidectomized animals also responded to adrenine. However, Mislowitzer failed to find this. Obviously more work is needed.

What part the nervous system plays in clinical hyperthyroidism is not clear. Sturm and Schneeberg[530] found that the iodine concentration in the medulla oblongata was much higher (34 to 89 gamma per cent) in those dying from hyperthyroidism than in the previously healthy (8 gamma per cent). Schittenhelm (1935) confirmed these findings, but it is not clear whether they indicate a causal relationship, or merely an effect.

CHAPTER IX

IODINE BALANCE

THROUGHOUT the many fluctuations which the thyroid and other tissues undergo in the course of the various physiological and pathological changes discussed in the preceding chapters, it is evident that if life is to continue for long periods the organism must maintain a certain minimal reserve of iodine. In other words, iodine balance must be preserved at very nearly the neutral point. Until recently such studies were extremely difficult technically. Consequently, when von Fellenberg in 1926 reported that he had analyzed ingesta and excreta in direct clinical metabolic studies and found that normally the organism is in iodine balance, this apparently naïve report was recognized in fact as a milestone in the progress of nutritional studies. Such observations still demand scrupulous attention to detail. For example, in a metabolic hospital ward false balances may be found because some medication, or some confection unexpectedly supplies relatively huge amounts of iodine which are unsuspected. An iodoform dressing on a surgical patient in an adjoining bed may supply sufficient extraneous iodine to spoil metabolic observations. Nevertheless, this type of study is important because it indicates clearly (*a*) when excessive amounts of iodine are drawn from bodily reserves, and (*b*) how much of the element must be supplied for daily maintenance under normal conditions.

This method is the most direct way of deciding the quantitative estimate of iodine requirements in health and in disease. There is another method which is more crucial but much less practical. Because many investigators agree with Marine that the most specific indication of iodine deficiency is hyperplasia of the thyroid, much effort has been spent to determine the minimal daily iodine required to prevent the histological reaction. A major difficulty is the fact that an iodine-deficient diet must continue for several months in children, and for several years in adults, before recognizable goiter appears.[131] Even with experimental animals under ideal conditions the results may be capricious. Thus, Jackson and

P'An[262] could not produce goiter experimentally with a poor iodine intake, although Drennan, Malcolm, and Cox[127] succeeded in doing so. By contrast Levine, Remington, and von Kolnitz[322] were able to state rather precisely that rats became goitrous on 0.14 gamma of iodine daily, but not on 1 to 2 gamma per day. Small amounts of iodine in the air, water and food may be sufficient to distort experimental results, which must perforce be continued for many months. For these reasons, greater stress has been laid upon measurement of the excretion of iodine by normal healthy adults, particularly in the urine; and upon the total iodine ingested in the natural diet of normal individuals. Indeed, Lunde[345] even suggested doubling the urinary output to estimate the intake of iodine, but subsequent carefully balanced studies have shown this procedure to be hazardous because other routes of excretion may behave erratically.

Because the fate of the iodine taken in depends upon (*a*) the amounts administered, (*b*) the route and rate of administration, and (*c*) the chemical form and other factors, it is evident that no simple formulation of iodine balance can be precise. Already, however, considerable data are available indicating the general trends in iodine metabolism, and we must content ourselves with scanning some of these.

Assimilation of Iodine

Cohn[91] studied the absorption of various inorganic compounds containing iodine after these substances had been administered enterally, and concluded that they were absorbed only after conversion to iodide. Consequently, because free elementary iodine and iodates must be converted into iodide ions before they can be absorbed, they act somewhat more slowly and slightly less effectively than inorganic iodide. These results, incidentally, throw some doubt on the superiority of liquor iodi compositus over potassium iodide as a readily assimilable form of iodine.

In two hours 80 per cent of the ingested iodide is absorbed from the small intestine and 63 per cent from the large intestine. Cohn found, furthermore, that the isolated stomach could absorb 31 per cent of potassium iodide in two hours. Free iodine was absorbed to the extent of 18 per cent after its conversion to iodide. Likewise Hamilton[217] studied the rate of absorption of labeled iodide (see

Chapter X) from the gastrointestinal tract in eight normal individuals. This was done by measuring the increment of radioactivity of the hand of each subject for two hours after ingestion. Apparently over four-fifths of the medication had been absorbed at the end of one hour. Similarly, Tiedcke [547] showed that the buccal mucosa could absorb iodide slowly, but repeated paintings over the course of several days are required to produce significant general systemic effects.

Assimilation of Organic Compounds

1. Diiodotyrosine. Both levorotatory and dextrorotatory forms are readily absorbed from the gastrointestinal tract. Little of the iodine (only a few per cent) is excreted in the feces, and this fecal excretion occurs even after subcutaneous administration.[1] For example, in adult rabbits receiving 10 grams of the dl- or l- compound over a period of several days, Oswald recovered the following iodines: of the dl- compound 46 per cent in the urine and 3 per cent in the feces; of the l- compound 89 per cent in the urine and 3 per cent in the feces. After administering large doses of racemic diiodotyrosine to rabbits, Foster and Gutman [177] found in the urine 60 per cent of diiodotyrosine, 10 per cent of inorganic iodide and 18 per cent of 3,5, diiodo-4 hydroxyphenyl lactic acid (i.e., a deaminated product). Using small doses, Elmer [140] and Elmer and Rychlik [150] recovered from 32 to 74 per cent of the iodine in one day's urine; smaller doses were more effectively retained in the body, at least temporarily.

After smaller doses, more inorganic iodine is found in the urine, indicating a greater relative degree of decomposition. Thus Elmer [140] found that after taking 0.2 gram of diiodotyrosine (containing 116 mg. of iodine) by mouth, his daily urine contained 57 mg. of inorganic iodide out of 86 mg. total iodine. There remained enough unchanged diiodotyrosine, nevertheless, to give the color reaction of Kendall and Osterberg.[277] The rapid absorption and decomposition of the compound was shown by the fact that even within an hour after ingestion the urine showed increased iodide. Similarly, Snapper and Grunbaum [515] found increases in salivary iodine soon after ingestion.

It was once thought that digestive enzymes might destroy diiodotyrosine by de-iodinating the substance when fed. Nevertheless, it was found[1] that even after subcutaneous administration the compound was decomposed in part to inorganic iodide before excretion. Furthermore, Harington and Salter[229] and Barkan and Kingisepp[30] found only very slight decomposition by pure trypsin after long periods. It must be conceded, however, that bacterial action in the intestine may partly de-iodinate the compound. Moreover, part of the circulating compound is excreted in the bile[298] and thus returned to the intestine, when it is again forced to run the gamut of bacterial action.

2. *Thyroxine and Its Derivatives.* Dry crystals of thyroxine are highly insoluble in Ringer's solution and can be dissolved conveniently in aqueous media only when very alkaline. This simple chemical fact was long neglected in therapeutics. Thus Schittenhelm and Eisler[489] recovered in the feces of man 86 per cent of the iodine administered by mouth in 6.5 mg. as solid thyroxine. When the same amount was introduced *in alkaline solution* into the duodenum of the dog, however, over 90 per cent was absorbed in from 2 to 12 hours. Indeed, thyroxine becomes increasingly soluble as the monosodium and disodium salts (formed by treatment with alkali) are produced by neutralization. This point was expounded quantitatively by W. O. Thompson, P. K. Thompson, Dickie, and Alper[544] by studies of calorigenic effect, and by Bøe and Elmer,[52] and Elmer and Rychlik,[150] who followed urinary iodine excretion. Nevertheless, Harington and his collaborators[191] and Salter's colleagues[472] had for years assayed thyroxine derivatives *in alkaline solution* purposely to take advantage of supersaturation, which occurs even in biological fluids *once* the crystalline hormone has been got into solution. Obviously, drugs should be administered in such a physical state as to favor assimilation, if their full physiological effect is to be realized. Thus Elmer and Luczynski[139] (see p. 147) found that the rabbit intestine absorbed over 85 per cent of *dissolved* thyroxine within 24 hours.

Thyroxine in Peptide Combination. Besides the formation of alkali salts, the addition of a peptide chain increases the solubility of thyroxine. Simple synthetic dipeptides of thyroxine are not very soluble,[23] but longer-chained compounds show increasing solubility and a greater tendency to supersaturation. Hydrolysis with acid produced the "jodthyrin" or "thyrojodin" of Baumann[40] and modern similar derivatives of "thyroidin" (desiccated thyroid). These are, in essence, peptones of thyroxine. As evidence of better solubility and absorption, Asimoff and Estrin [25] found in the urine of animals from 63 to 95 per cent of the iodine administered *per os* as "thyroid," in contrast to only 5 to 9 per cent for thyroxine.

Likewise, Harington and Salter [229] made a polypeptide of thyroxine by tryptic digestion of thyroglobin. The effect of this compound when administered by mouth was practically the same as when injected intravenously. Furthermore, it reproduced, *in terms of iodine*, the same effect as intravenous thyroxine.[472] Lerman and Salter [318] have studied in athyreotic humans the calorigenic effects of human thyroglobin, its peptones, its peptides, as well as d-, l- and racemic thyroxine. In terms of iodine, these compounds are all equally effective under clinical conditions, provided they are administered under appropriate conditions favorable to absorption. Even the artificial thyroid protein (plastein) made by Salter [467] gives a similar quantitative result.

There is no doubt that whole thyroid substance is very efficiently assimilated by the gastrointestinal tract.[417] It is not certain, however, how far the intestinal enzymes break down the thyroglobin when whole thyroid or its protein is administered. The problem is perplexing because whole thyroid produces in myxedematous patients a greater effect than the thyroxine which it yields,[229] as explained elsewhere. Barnes and Bueno [33] presumed that it is partially absorbed as a long-chained peptide or proteose, because they obtained positive precipitin reactions in the blood after ingestion of thyroid.

Iodine Requirements

It is evident from the magnitude of iodine elimination under the various conditions just considered that in hyperthyroidism the organism may require several times the normal intake. Although such patients frequently develop acutely a rather marked depletion of their reserves, this deficit ordinarily is soon remedied by the heroic doses commonly used in the clinics of today. As will be shown in Chapter XI, such medication is many times the normal requirement and far in excess of the need even in disease. It is much more difficult to assess normal values to be used in compiling dietaries for large groups of individuals, or even whole populations made up of heterogeneous elements. In general, however, experts in nutrition and professional dietitians feel that they should know approximate values for two vaguely defined states. These are, first, the *minimal normal requirement* which will barely prevent detectable pathological change and, secondly, the *optimal normal requirement*, an increase in which will result in no gain in health, efficiency, or "joie de vivre."

Minimum Normal Requirement. Values for the minimum daily requirement of iodine vary between 40 micrograms [131] and 15 micrograms.[418] Smaller rations, if continued, produce signs of iodine deficiency. The simplest criterion of such deficiency is hyperplasia of the thyroid, which Marine and associates [359] consider a very important sign of deficiency disease. Because the organism has a reserve of iodine in the thyroid and elsewhere, several months may elapse before a deficient diet produces this histological change. Furthermore, even bad diets usually contain a little iodine. Obviously, then, the above estimates are very crude. Likewise pregnancy, growth, and conditions involving a high caloric turnover increase the requirement, which is a relative quantity. For example, Eggenberger [131] believes that the growing child requires at least 1 microgram per kilogram.

Optimal Normal Requirement. Estimates of the amount of iodine in the diet of normal healthy adults in various parts of the world vary from 15 to 500 micrograms daily. Obviously, measurement of intakes gives only a rough idea of bodily need. More useful are the data of Cole and Curtis,[96] who found that the daily iodine retention in normal subjects consuming varying quantities of iodine was from 15 to 25 micrograms, and they suggest that this amount represents the optimal requirement. On the other hand, values for urinary iodine in the literature, i.e., about 50 micrograms, suggest that the optimal requirement should be at least 100 micrograms. The maintenance requirement of thyroxine iodine in athyreotic patients is nearly 200 micrograms of iodine per day,[390] but some of this hormonal iodine may be trapped and used again.

In summary, it seems likely that a normal adult man will remain healthy on 200 micrograms iodine a day, and possibly on one-eighth of this amount.

For growth, larger amounts are needed when expressed in terms of kilogram of weight or square meter of body surface. Babies take in from 20 to 44 micrograms per day [151] and older children about 50 micrograms daily.[418] Urinary studies by Curtis and Puppel [116] also show a relatively high iodine excretion in the young.

Metabolic studies of iodine balance show that the organism can compensate for wide variations in iodine intake. Thus Fellenberg [168] found that when 17 micrograms of iodine were taken in, 15 micrograms were put out. This "acute" observation represents equilibrium within the experimental error. About 40 per cent of the excreted iodine appeared in the urine, and about 26 per cent in the feces. The nose, skin, and lungs contributed, respectively, 11, 9, and 14 per cent. On more liberal intakes, namely, 60 to 110 micrograms per 24 hours, Scheffer [480, 482, 484, 483] found a tendency to negative balance, as shown in Table 36. In the absence of profuse sweating, over half the iodine was eliminated in the urine. Inconstant excretion by lung, in addition to sweating, complicates the picture. Fur-

TABLE 36

IODINE EXCRETION IN HYPERTHYROIDISM

Diagnosis	Basal metabolism	Daily intake of iodine in micrograms	Daily Excretion of Iodine in Micrograms				
			Urine	Feces	Skin	Lungs	Total
Hyperthyroidism							
six cases	..	80.0	2.5	38.8	14.4	...	55.7
	..	80–100	9.0	67.8	0	...	76.8
	..	150.0	93.7	34.9	20.5	...	149.1
	..	80–100	25.4	49.7	16.0	...	91.1
	..	80–100	8.9	60.5	2.1	14.0	85.5
	..	100–120	15.6	66.6	27.0	17.3	126.5
Hyperthyroidism single patient							
Before x-ray	..	80–100	25.4	49.7	16.0	...	91.1
After x-ray	..	80–100	19.4	71.6	0	...	91.0
After x-ray	..	100–120	7.7	130.4	0	...	138.1
Typical Graves' disease, single patient							
April 12	+54	93.5	265.0	24.0	0	...	289.0
May 24	+55	75.0	58.0	61.2	0	...	119.2
June 22	+55	108.0	30.2	39.1	0	...	69.3
July 10	+29	129.0	25.5	49.4	53.4	...	126.5
Cardiovascular disturbance simulating hyperthyroidism	..	50–70	11.2	7.5	24.4	...	43.1
	..	50–70	25.9	19.6	11.3	...	56.8
Normal control							
1932, V.	..	130.6	57.1	2.8	44.8	...	104.7
1932, VI.	..	53.9	15.3	5.0	29.2	...	49.5
1932, XI.	..	155.0	116.1	...	23.7	Traces	139.8
1932, XII.	..	....	27.0	...	68.4	4.3	99.7

After: L. Scheffer, Klin. Wchnschr. *12*: 1285. 1933, and *13*: 1571–2. 1934.

thermore, as Cole and Curtis [96] showed, as much as one-fourth of the excretion may be fecal on occasions.

In cattle, Fellenberg [168] found quite different results. Not only is the turnover ten times that of man, but the fecal excretion is severalfold greater than that in urine or in milk. It should be remembered that 1 kilogram of hay may contain 400 micrograms of total iodine. Albino rats were found by Cole and Curtis to remain in iodine balance on intakes of from 3 to 14 micrograms daily, as judged by mixed urine and fecal excretion.

The normal iodine requirement is increased, of course, by factors which augment total metabolism. Thus, exercise causes an increase in blood iodine within a few minutes, and subsequently, urinary excretion may be double the control value provided excessive sweating does not divert iodine elimination to the skin, as often happens.[480] Elmer [139] (see p. 140) presumes that the increased blood iodine is due to thyroid secretion.

Diet and Iodine Deficiency

Because simple goiter is thought by Marine [355] and others to be caused either by *absolute* or *relative* iodine deficiency, it is obvious that the prevalence of iodine in the flora and fauna of various geographical regions may have a significant influence on public health. Long before it was known that iodine occurs in the thyroid, Chatin [83] showed that the development of goiter is related to decreased iodine intake in drinking water, food and in air. "Tales sunt aquae quales terrae, per quas fluunt," as Pliny the Elder said. He concluded (*1*) that goiter and cretinism are rare in districts which are rich in iodine; (*2*) they do occur frequently, however, in districts where iodine is poor in natural products; and (*3*) iodine is a specific preventative of goiter. Since then many analyses of drinking water have been made, some of which are summarized in McClendon's recent book.[377] It is impossible to cite here much of the data reviewed by McClendon to show correlation between the incidence of goiter and iodine in water. Fellenberg,[169] for example, found that Effingen had 1 per cent goiter incidence and 2.3 gamma per cent in drinking water, but Hunzenschwil and Kaisten had 56 to 62 per cent goiter incidence and only 0.04 to 0.8 gamma per cent iodine in water. Soil analyses are summarized in Table 37. Reith [455] studied Campen (Holland), where goiter was nearly unknown until a hygienic water supply increased goiter incidence to 41 per cent. The former, unsafe water contained 10 to 20 gamma per cent iodine, as against 1 gamma per cent iodine in the pure water.

Of course, water is not the only factor, because imported foods high in iodine content [314] may protect the inhabitants of *virtual* goiter districts. Likewise, local samples of water may vary considerably and mislead the investigator unless several sources are averaged. Crabtree and Mason add the possibility that the mode of combination of the iodine in the ingesta is more important than the absolute total amount, but this point is probably not often of great importance. At any rate, it is clear that vegetation growing in iodine-rich soil shows a high iodine content. This holds for France (Chatin, 1850), Switzerland (Fellenberg, 1924), New Zealand (Hercus, Benson, and Carter, 1925), the Argentine (Mazzocco, 1929), and the United States (McClendon, 1939), although of course there are individual exceptions. Fellenberg [168] found that the Japanese "kombu" might contain 2.3 mg. of iodine per kilo. Indeed, McClendon [377] found that in mountainous Kitakami and Chichibu, where no seaweeds are eaten, goiter is present. The rest of Japan is nearly free of goiter, presumably because seaweeds are a constant constituent of the Japanese diet.

TABLE 37

IODINE CONTENT OF SOIL AND GOITER RATES IN SWITZERLAND

Locality	Percentage of goiter	Iodine (γ/kg.)
Effingen	1.0	11,900
Hornussen	12.1	4,940
Hunzenschwil	56.2	620
Kaisten	61.6	1,400

From: J. F. McClendon, Iodine and the Incidence of Goiter, Univ. of Minn. Press. 1939, p. 11.

Inasmuch as the air is in contact with soil and water, its content also varies in fairly close parallel with the percentages in the latter. In goitrous Alpine districts the iodine in the air may fall to 0.04 gamma per milliliter of air. In the Kreuznach valley, for example, the concentration was 6.2 gamma iodine per cubic meter of air, amounting to 12 or 40 micrograms in the inspired air daily. Likewise rain or dew may carry iodine. Obviously, the type of food is highly important.

Conditioned Iodine Deficiency. Scheffer has described a "conditioned" deficiency state in which goiter arises on an adequate intake of iodine. The reason is faulty absorption by the intestine. Similarly, the Mellanby's found that a diet rich in unsaturated fats produced goiter in puppies. Marine[354] pointed out that intestinal parasites may utilize iodine and so abstract it from the net intake. Indeed, in the goitrous cases of Scheffer and Megay[484] iodine turnover appeared small. For example, on a normal iodine intake (80 to 100 micrograms) patients with goiter had a low urinary excretion (3 to 9 micrograms daily).

Vitamins and Iodine

Vitamin A. Fasold believes that vitamin A should be regarded as a controlling agent of thyroid function. There is, indeed, considerable evidence that this vitamin acts antagonistically to the thyroid hormone. For example, large doses of vitamin A partly inhibit the effect of thyrotropic hormone in guinea pigs. Furthermore, thyroidectomized goats excrete carotene, whereas only vitamin A appears in normal goat milk. In general, iodine metabolism varies with the degree of thyroid activity or of thyrotoxicosis attained after a new equilibrium is established. Chidester (1932), however, suspects that the effectiveness of vitamin A varies with the amount of iodine introduced accidentally with the vitamin, and this factor must be eliminated before a final judgment can be given (Schneider, 1938).

Vitamin B Complex. In beriberi Takasugi found at first a marked increase in blood iodine and in basal metabolic rate; later both dropped. The last stages of vitamin B deficiency show a resting gland histologically and a decreased hormone content. Indeed, rats develop a sort of colloid goiter if their diets lack both iodine and vitamin B_1. In man, however, vitamin B_1 generally has little influence upon the thyroid status. (Cf. Carpenter and Sharpless, 1937.)

Vitamin C. In experimental scurvy in guinea pigs the thyroid shows a picture of hyperfunction. Indeed, Fasold (1934) found that in C-deficient animals the blood iodine may increase two- or threefold. Conversely, Löhr[342] found that in both normal and thyrotoxic organisms ascorbic acid in excess reduces the blood iodine (without affecting the basal metabolic rate). Marine and Rosen,[362] and Elmer[139] (see p. 337), found that ascorbic acid in large doses may inhibit the action of thyrotropic hormone. No data on iodine excretion are available. In general, there seems to be a reciprocal interaction between thyroid activity and both the requirement and the anti-thyroid effect of vitamin C.

Vitamin D. In avitaminosis-D in young rats, Krauss and Monroe (1930) found the iodine content in the thyroid to be decreased. Although the morphological picture of the thyroid often is that of hyperfunction, the blood iodine level in human rickets is normal. Moreover, there is no direct effect of vitamin D on blood iodine. No good data on iodine output are available. (See p. 103.)

Relative Iodine Deficiency. Obviously, the minimal need for iodine varies. More hormone is needed in puberty, pregnancy, lactation and the menopause, — critical periods for the development of clinical goiter. Likewise, the requirement is increased by intercurrent causes, such as infections, disturbances in endocrine and vitamin metabolism, and "toxic" factors, e.g., oatmeal in the diet of mice and cabbage in that of rabbits. Biochemical phenomena may intervene, e.g., a disturbed ratio of calcium to iodine, of fluoride to iodide, or of other constituents. Alimentary failure of absorption may likewise vitiate estimates of normal iodine intake.

A few examples may be given. In pregnancy, inadequate iodine supply may lead to hyperplasia of the fetal thyroid as well as of the maternal thyroid, particularly in the last months of gestation when considerable stores of iodine are being laid down in embryonic tissues. After birth some 20 micrograms of iodine must be supplied daily in the milk, else the baby will develop goiter.[151] Indeed, Schmelling [498] prevented thyroid hyperplasia in rats, and Davis prevented it in mothers and children, by giving small doses of iodine during pregnancy and lactation.

Marine and his associates have studied the cabbage goiter of Chesney.[84] The presumable cause is an organic cyanide (e.g., methyl cyanide) which depresses tissue oxidation unless excessive amounts of thyroid hormone are available. Most striking is the prophylactic effect of iodide therapy in these states, unless the "toxic" substance is present in too large amounts. In the Carpathians, V. Suk (1931) has reported goiter in people who live chiefly on cabbage together with oatmeal, bread and potatoes. Starr [517] likewise has studied a similar effect in patients treated with thiocyanate for long periods.

Elsewhere it is indicated that, according to Marine, vitamin C may prevent goiter. Lack of vitamin A was found by Coplan and

Sampson to induce thyroid hyperplasia in female albino rats. Similarly, McCarrison found "lymphadenoid goitre occurring in rats fed on diets containing an insufficiency of vitamin A."

In man, Puppel and Curtis [442] have found that calcium varies inversely with iodine balance. Similarly, Tanabe produced goiter in rats within from three to five months with a low-iodine, high-calcium diet; and by adding iodine he prevented thyroid hyperplasia. Such findings were construed as implicating the hard water frequently found in goitrous districts. Nevertheless, Remington and Levine [456] and others have found no effect by varying the calcium-iodine ratio, and it seems likely that even though iodine influences calcium metabolism, the converse is not true generally. (Cf. Hellwig, 1940.)

Kraft and May [296] have advanced a similar point of view for fluoride. They found the fluorine concentration to be high (22 to 29 gamma per cent) in goitrous regions, as compared with 14 gamma per cent (normal).[163]

Prophylactic Dosage of Iodine. Kimball [279] has pointed out that a great argument in favor of iodine lack as the precipitating cause of simple goiter is afforded by the response of school children to iodine therapy. In Cleveland, from 1924 to 1931, the incidence of goiter dropped from 36 per cent to 2 per cent. Because the fear of iodothyrotoxicosis still prevails, there is much discussion as to the size of the daily prophylactic dose. Elmer [139] (see p. 499), recommends from 50 to 100 micrograms daily; Means [384] suggests 1125 micrograms. Marine also suggests that desiccated thyroid increases the effectiveness of iodide. For therapy, Eggenberger [131] says the dose should be from 80 to 250 gamma iodine, although not all goiters will recede because of extensive structural changes.

Physiological Excretion

Urinary Iodine. Urinary iodine excretion depends upon the iodine intake in food, water, and air. Thus, in a district with 0.09 gamma per cent in drinking water, the 24-hour excretion was 47 micrograms, whereas in another district with 8.9 gamma per cent in the water it was 185 micrograms.[455] Consequently, it is more profitable to study excretions in districts where water and flora contain only moderate concentrations of iodine. Under stable conditions in such districts, individual variation is small, — not over 10 to 15 micrograms in 24 hours. Normally, about half of the total iodine leaves the body by way of the urine.[96] A few representative observations follow.

In Columbus, Ohio, nine-year-old children excrete approximately 41 gamma daily and adults 50 gamma.[116] In Lwów, values below 20 gamma are found only in goitrous individuals, and values above 50 gamma frequently accompany thyrotoxicosis[139] (see p. 114). Nevertheless, 70 gamma occasionally may be found in the urine of normal individuals. In Danzig, Isenbruch[261] found 6 gamma per cent iodine concentration in the urine of nursing infants. Goats[106] excrete 21 gamma daily and dogs[526, 528] between 8 and 67 gamma, depending on the animal's size. In cattle enjoying an iodine-rich fodder, however, urinary excretion may reach 600 gamma.[476] According to McClendon,[378] twice as much iodine may be excreted by day as by night. Indeed, 24-hour urine samples are essential in careful studies of iodine metabolism because of hourly variations.[480] No doubt diurnal assimilation of exogenous iodine is largely responsible for such fluctuations. In fasting animals excretion is more uniform, and declines but slowly.

Most, if not all, of the urinary iodine is present as iodide. No thyroxine can be detected in the urine,[154] but whether any diiodotyrosine is present normally is not yet clear.

Fecal Iodine. Because almost no iodine is excreted in the feces of fasting men, it seems clear that fecal iodine is almost entirely of exogenous origin. When large doses of thyroxine are administered intravenously the large bowel actively excretes part of the iodine.[493] The bile also contributes to fecal iodine, because its iodine content may increase from 9.2 gamma per cent, fasting, to 45 gamma per cent after feeding.[146, 147] A large part of the biliary iodine is reabsorbed so that there is a "circulation" of iodine analogous to the "circulation" of bile salts. Of course, unabsorbed iodine in food stuffs also contributes to the fecal iodine concentration. Consequently, in diarrhea more iodine is eliminated, along with unabsorbed food stuffs, whereas in constipation relatively little iodine is eliminated. On a routine diet, Scheffer[480, 483] found only from 2 to 10 gamma iodine per 24 hours, namely, 3 to 9 per cent of the total iodine excretion. Cole and Curtis[96] found variable but larger amounts, i.e., from 6 to 27 per cent of the intake.

In animals, fecal iodine appears to be small, as judged from the following values for 24-hour excretion: for the hog, 1.8 micrograms; [476] for the goat, 3 micrograms; [106] for the rabbit, 9 to 12 micrograms [139] (see p. 119). On the contrary, in cows eating large amounts of iodine-containing fodder, Fellenberg [168] found as much as 1300 micrograms per 24 hours.

The iodine excretion of normal individuals is increased by such factors as exercise, which augment total metabolism. After exertion, urinary excretion may reach double the control value,[378] provided excessive sweating does not divert iodine elimination to the skin, as often happens. It is possible that the increased blood and urinary iodine is due to thyroid secretion [139] (see p. 140). Kommerell [293] found no increase in basal metabolism after exercise in thyroidectomized animals. This phenomenon deserves further study, in view of the prevailing opinion that the thyroid exerts its effect only after a latent prodromal period.

Other factors which increase total metabolism also increase iodine elimination. For example, it is said that mental excitement alone may double the excretion.[168, 579] Likewise, a fever of 39°C. may treble the excretion.[168] Very high iodine excretions — as much as 3000 micrograms daily — have been observed after severe operations like thoracoplasties,[114, 111] and there is an associated hyperiodemia which begins within a few minutes after operation and lasts for several days. McCullagh [381] believes that this extra iodine comes from the thyroid, whereas Curtis and Phillips believe it to be of extrathyroidal origin. The high iodine elimination of thyroid disease will be discussed in another section.

Excretion by Other Routes. The iodine in milk, in sweat, and in saliva has been discussed in Chapter IV. Under normal living conditions near the seacoast, these media play a minor part in the total iodine excretion. Courrier and Aron [104] believe that the thyroid hormone can be transmitted through the milk of bitches and so affect the histological status of the thyroids of puppies. The possible amount of thyroxine so transmitted has been estimated by chemical analysis and, if present at all, must be exceedingly small.[151]

HUMAN IODINE BALANCE

Thus far three careful studies of iodine balance in normal human subjects have been made, namely, those of von Fellenberg,[168] of Scheffer,[480] and of Cole and Curtis.[96] Before summarizing these results, it may be profitable to outline the metabolic procedure used by the last investigators as representative of the general method. Normal subjects or patients were selected and their past history scrutinized to exclude iodine medication for two months previously. The subjects were provided with loosely woven cotton underwear, previously washed in doubly distilled water. Each day the subjects were bathed in six liters of distilled water, and dried with towels which had been washed in doubly distilled water. Then underwear and towels were rinsed, first in this water, and then three times in fresh distilled water, bringing the total volume of ablution fluid up to ten liters. An aliquot of this fluid was saved until three such were on hand, when all aliquots were combined, because the observations were conducted on the basis of time intervals of three days each. Each day the urine, carefully collected throughout the 24 hours, was made up to two liters, and eventually three-day samples were combined for analysis. Fecal elimination was separated in point of time by feeding carmine pigment every third day half an hour before breakfast. The feces were subdivided, dried, and powdered for analysis.

The subjects were maintained at bed rest and at constant weight on a diet which remained unchanged day by day throughout the weeks of observation. Uniformity in the food was attained by using canned foods bought in a lot sufficient for the entire series of observations. Likewise, meat and potatoes sufficient for the entire experiment were reserved at one time, and a single homogeneous lot of butter was used. Daily samples of the food were weighed out, dried, and three-day samples combined, ground, and supplemented with salted butter and sugar appropriately. Aliquots of this mixture were then analyzed. Butter and water were tested individually.

By such minute precautions it is possible to attain balances within 10 to 20 micrograms per day for a man taking in nearly 200 micrograms daily. With smaller intakes, the error is less, and with larger intakes it may be greater. The necessity for such complicated precautions is evident from the fact that the combined urine and stools may account for less than 50 per cent of the iodine intake, even on such a monotonous diet. Sweating is the major route through which iodine excretion may be diverted, thus to reduce urinary iodine. Likewise, diarrhea may increase fecal iodine elimination and thus reduce urinary elimination.

Observations of von Fellenberg. This investigator studied his own iodine balance on four consecutive levels of intake, i.e., 17, 55, 75, and 80 micrograms of iodine per day. He consumed the same basal diet throughout the observations, and analyzed urine, feces, sweat, and nasal secretion. The results of these four successive states may be summarized as follows.

I. Daily intake 17 micrograms. Average total excretion per day 14 micrograms, or 84 per cent, chiefly urinary. Sweat and nasal secretion 15 per cent.

II. Potassium iodide added to basal diet to make the total intake 58 micrograms per day. Total daily output averaged 38 micrograms, or 67 per cent of the ingested; 24 micrograms were in the urine, and 6 in the sweat and nasal secretion.

III. Cod liver oil added to the basal diet to make the total intake 75 micrograms per day. The daily excretion averaged 37 micrograms, i.e., 50 per cent of the ingested. The nasal and sweat secretion accounted for about 10 per cent; the urine for about half of the excretion.

IV. Sardines or water cress added to the diet to make the daily iodine content 80 micrograms of iodine per day. The urinary iodine averaged 80 per cent of the ingested. The nasal and sweat excretion was unaltered. Fecal iodine was only slightly greater than in observation I.

V. The basal diet of observation period number I was resumed. After five days the subject had not yet reverted to the initial balance.

Observations of Scheffer. Similar balance studies were made by Scheffer on 12 normal subjects, at five levels of iodine intake, namely: 54, 59, 66, 130, and 155 micrograms per day. The metabolic balance was derived from summated excretions (urine, feces, and sweat) over balance periods of three or five days. The results may be summarized as follows.

I. On an intake of 54 micrograms per day over 90 per cent of the intake was accounted for in the excreta. In one subject about 60 per cent appeared in the urine, 20 per cent in the sweat, and only 6 per cent in the stool.

II. On about 65 micrograms of iodine per day, the combined excretions of seven subjects ranged from 30 to 44 per cent of the intake.

III. On an intake of 130 micrograms per day, the total excretion of one individual was 105 micrograms, i.e., 81 per cent of the intake.

Of this amount 57 micrograms appeared in the daily urine, 45 in the sweat, and only 3 in the feces. Thus urine and feces together accounted for only 46 per cent of the intake.

IV. On a daily intake of 155 micrograms, a single individual excreted 116 micrograms in the urine, 24 in the sweat, and only about 5 micrograms in feces. In this case the urine alone contained 75 per cent of the ingested material.

The most constant fact which emerges from these rather variable results selected from Scheffer's detailed data is the low fecal iodine. This is important because Scheffer found from studies on seven individuals with goiter accompanied by hyperthyroidism, and on six additional patients suffering from Graves' disease, that fecal [481] iodine was several times higher than the normal value. In Graves' disease also, increased sweating led to increased elimination of iodine through the skin. Despite the extra iodine thus diverted from the total metabolism, the iodine in the urine tends to be higher than normal. In short, hyperthyroidism entails increased elimination through multiple channels. By contrast, in cases with goiter without hyperthyroidism, the urinary output was regularly decreased; but sometimes even with hyperthyroidism the urinary output might be decreased. In goiter alone, elimination through the lungs might be increased. In Table 36 are presented such values for convenient comparison with the normal values obtained by Scheffer, although these pathological balances will be discussed later in this chapter. Similar data of Puppel and Curtis are reproduced in Fig. 26.

Observations of Cole and Curtis. These investigators studied two normal subjects (A and B), and a third subject (C), with essential hypertension. In addition they studied two subjects (D and E), suffering from hyperthyroidism. All of these subjects were maintained on a monotonous diet already described. In general, the results were in fairly good agreement with those of von Fellenberg and Scheffer except that fecal iodine was somewhat higher. They found that at times less than 50 per cent of the intake may appear in the urine and feces combined. Some of the details follow.

Subject A, white male, age 28 years. Received 156 micrograms of iodine daily. The average daily excretion was 47 micrograms in the urine, 27 in feces (totaling 74 or 47 per cent). The negative balance was therefore 82 micrograms. Similar observations on another normal male are given in Table 38.

Subject B, white female, age 31 years. Ingested 56 micrograms of iodine daily. The average output daily was 35 micrograms in the urine, 9 in feces, totaling 44 micrograms daily, or 79 per cent of the intake.

Cole and Curtis point out that subject A had a continuously moist skin and was restless, whereas subject B had a dry skin and remained

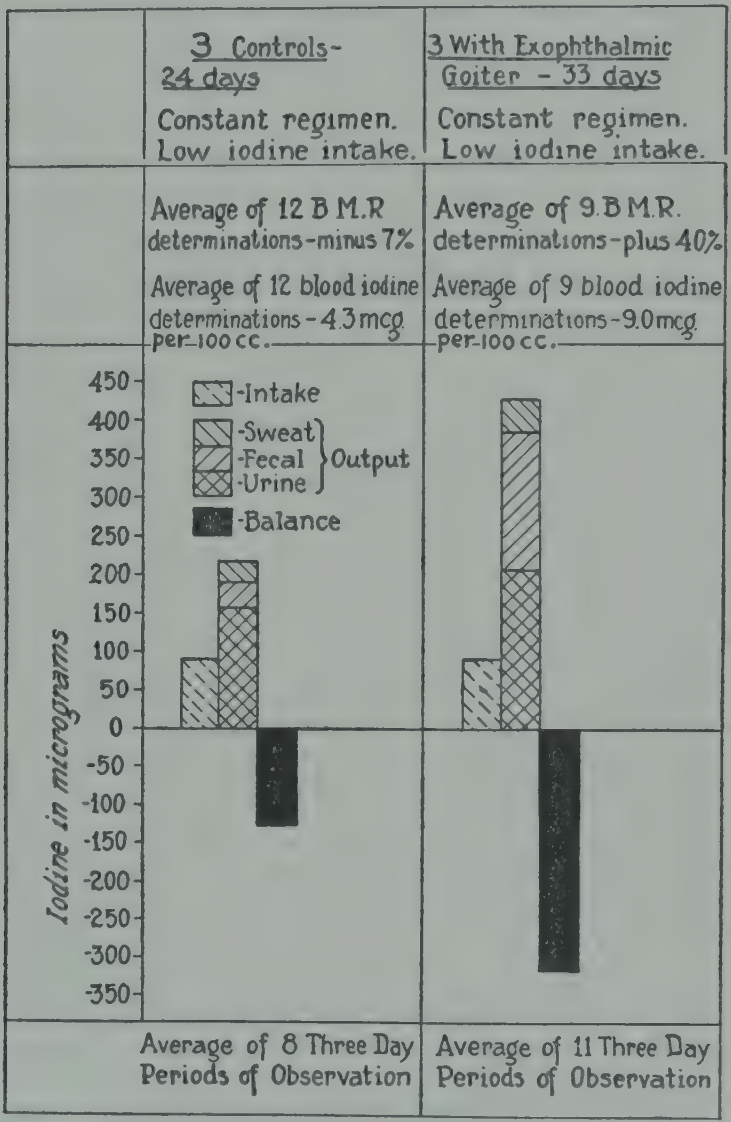


FIG. 26. Average iodine balances of three normal individuals (at left) compared with average iodine balances of three patients with exophthalmic goiter (at right). Even in the normal individuals, the excretion of iodine was greater than the intake. In the hyperthyroid individuals this negative iodine balance was increased nearly threefold, largely due to increased fecal elimination.

From: I. D. Puppel and G. M. Curtis, Arch. Path. *26*: 1111. 1938.

TABLE 38

DATA FOR J. R., A NORMAL WHITE MAN AGED 56

			IODINE									
			Output									
Period	Date when started 1936	Weight, kg.	Urine mcg.*	Feces, mcg.	Sweat, mcg.	Total, mcg.	Intake, mcg.	Balance, mcg.	Date, 1936	Blood Iodine mcg. per 100 cc.	B.M.R. percentage	Comment
Diet constant, totaling 2,000 calories and 80 gm. of protein												
1	10/15	59	153	41	28	222	77	−145	10/15	4.6	−10	Rest in bed
									10/17	4.0	−14	
2	10/18	59	153	30	21	204	74	−130	10/19	3.8	−13	
									10/21	5.3	−11	
3	10/21	59	157	32	26	215	61	−154	10/23	4.5	−16	
Diet supplemented with iodized milk												
1	10/24	59	1,010	102	23	1,135	1,608	+473	10/27	6.9	−13	Iodized milk given, 750 cc. daily
2	10/27	59	1,340	97	29	1,466	1,698	+232	10/29	3.8	−11	
Diet supplemented with iodine as potassium iodide												
1	10/30	60	5,980	51	47	6,078	9,078	+3000	10/30	6.4	−13	Iodine administered, 3 mg. daily, from October 30 to November 5
2	11/2	60	7,200	66	70	7,336	9,078	+1742	11/2	9.3	−7	
3	11/5	60	14,600	111	112	14,823	18,078	+3255	11/5	17.3	−8	Iodine administered, 6 mg. daily, from November 5 to 11
4	11/8	59	14,300	181	123	14,604	18,078	+3474	11/8	15.0	−7	
									11/11	16.6	....	Iodine administered, 12 mg. daily, from November 11 to 14
5	11/11	59	26,600	173	193	26,966	36,078	+9112	11/14	29.6	....	

* A microgram (mcg.) equals 0.001 mg.

From: I. D. Puppel and G. M. Curtis, Arch. Path. *26*: 1097. 1938.

quietly in bed. Unfortunately, the excretion of sweat in these two subjects was not studied.

Subject C, white female, age 38 years, with essential hypertension. The intake was 39 micrograms of iodine daily; the output averaged 22 micrograms in the urine, 10 in the stool (totaling 32 micrograms, or 82 per cent), and 19 in the sweat. Thus the negative balance was 12 micrograms per day.

Subject D, white female, age 24 years, suffering from Graves' disease. The basal metabolic rate was plus 28 per cent. When the intake of iodine was 162 micrograms daily, the output was partitioned as follows: 46 micrograms in the urine, 94 in the stool (totaling 140 or 86 per cent), and 15 in the sweat. Thus the total excretion was 155 micrograms daily with a positive balance of 7. When the intake was 147 micrograms daily, the output was partitioned as follows: 46 micrograms in the urine, 97 in the stool (totaling 143, or 97 per cent), and 27 in the sweat. Thus the total excretion was 170 micrograms daily, with a negative balance of 24.

Subject E, white female, age 24 years, suffering from goiter with hyperthyroidism. Basal metabolic rate was plus 65 per cent. When the intake was 158 micrograms daily, the urine contained 116, the feces 43 (totaling 159 or 101 per cent), and the sweat 23. Thus the combined excretion was 182 micrograms daily and the patient was in negative balance to the extent of 24 micrograms. When the intake was increased to 336 by the addition of potassium iodide to the basal diet, the excretion was partitioned as follows: 172 micrograms daily in the urine, 237 in feces (totaling 409 or 122 per cent), and 18 in the sweat. Thus, the combined daily excretion was 427 micrograms, indicating a negative balance of 91.

The large negative balance in this last case may possibly be the result of inunctions of the neck which the patient had stopped after two months of use, a fortnight before entering the hospital. It is evident, however, that both subjects D and E showed a negative balance and it is entirely conceivable that depletion (*a*) of the thyroid reserve, and (*b*) of the wasting skeletal muscles might account entirely for the high loss of iodine. The high fecal iodine encountered in these two patients with hyperthyroidism is characteristic and will be commented upon later. It is presented here for purposes of convenient comparison.

Fasting Iodine Excretion. Fasting has relatively little effect on the total excretion of iodine in a well nourished individual. To be sure, the fecal iodine rapidly disappears; only 0.4 microgram per day is found. The iodine reserves, however, maintain a normal urinary excretion for many days or weeks, depending on the original richness of the diet in iodine. The average "endogenous" urinary iodine excretion was found by Sturm [526] to be 18 micrograms in dogs which had fasted for four days. In thyroidectomized dogs, however, fasting produces a rapid, marked drop, and the total excretion may approach zero. Presumably, the greater part of endogenous iodine elimination is linked up with thyroid activity, directly or indirectly. Of course, after a depletion of iodine reserves by fasting, restoration of a normal diet is accompanied by a temporary retention which causes a negative iodine balance.

Pharmacological Effects of Administered Iodine

Although it has been demonstrated that the dermatitis of iodism is accompanied by abnormal elimination of iodine through the skin,[419] this situation must be highly abnormal. After the administration of potassium iodide, diiodotyrosine, or thyroxine, approximately 90 per cent of the iodine in question eventually can be found in feces and urine.*

Iodide

The administration of iodide produces no increased elimination in the expired air.[168] Increase in salivary iodine has already been commented upon; the absolute amount excreted is very small. In lactating animals the milk likewise contains more iodine,[477] but, like saliva, it accounts for only a few per cent of the extra iodine. Scharrer [475] studied this effect in lactating goats. In 30 minutes after oral administration of 7.5 mg. of iodide the concentration in the milk had increased twentyfold. Six hours later it reached 73 gamma per cent, and resumed normal levels only four days later. The iodine is in the skimmed milk fraction. In cows, likewise, a small percentage of the extra iodine appears in the milk.[168, 435] Similar results were found by Fellenberg in lactating women.

Although under strictly physiological conditions iodine leaves the body chiefly as inorganic iodide in the urine, when therapeutic (or toxic) doses of iodine-containing compounds are given, excretion either by the kidney or by the intestine may predominate, according to circumstances. Ordinarily, the brunt of the excretion of extra iodine administered is borne by the kidney. To a lesser extent the bowel participates, partly by virtue of biliary secretion.

---

* Szász reported a striking finding with diiodotyrosine, i.e., that over 16 mg. were excreted by the kidney, as against 59 gamma by the skin.

Renal Elimination. Iodide, given by mouth in a single dose of 2 grams, increases the urinary iodide markedly for 48 hours and affects urinary iodide excretion for over a week. As much as 92 per cent of the added iodide can be recovered in the urine in eight days.[1] When small doses of iodide are given (i.e., under 0.1 gram) a higher percentage is retained, especially in the thyroid.[150] The peak of the iodide excretion is then reached in less than six hours, and with these smaller doses urinary iodine begins to approach normal after 24 hours [139] (see p. 170). Between 21 and 32 per cent of the small dose administered appears in the urine on the first day. This fraction may be altered in thyroid disorders, as described in Chapter XI.[137]

As regards the elimination of iodides by the kidney, only a small part is excreted by filtration through the glomeruli; [185] most of the iodine is contributed by active tubular secretion. Functional tests of the kidney actually have been devised on this basis. For example, a patient with chronic nephritis, after ingesting 1 gram of potassium iodide may excrete only 15 to 20 per cent of the iodine in the urine, as against 70 per cent for a normal man.

Fecal Elimination. After the administration of iodides, by mouth or parenterally, mere traces are eliminated by the bowel of dogs and man.[1] In intestinal diarrhea, however, larger amounts may be excreted,[168] up to perhaps one-fourth of the extra iodine. In cows, on the contrary, considerable amounts of the extra iodine may appear regularly in the feces.[436]

Organic Iodine Compounds

Renal and Fecal Elimination. When diiodotyrosine is given in large doses by mouth, much of the iodine appears in the urine, i.e., 89 per cent in rabbits,[420] 66 per cent in dogs,[1] and a like quantity in rats.[177] In man, Elmer [150] recovered 32 per cent on the first day. Much of the iodine so eliminated represents diiodotyrosine decomposed in the blood and tissue, rather than pre-existing iodide. This is inferred from the lag and slow elimination of this iodine as compared with potassium iodide.[150] About 20 per cent of the iodine is recovered in the feces of dogs,[1] about 3 per cent in rabbit feces,[420] and 10 per cent in rat feces.[177] After the intravenous injection of diiodotyrosine, only a few per cent are recovered from the feces; accordingly, urinary iodine elimination is slightly higher than after oral administration, namely 76 per cent as against 66 per cent.[1]

Thyroxine. The elimination of iodine after the feeding of thyroxine or its derivatives varies with the solubility of the preparation used.[544] For example, when a man ingested 2 mg. (i.e., 1300 gamma iodine) *in alkaline solution,* 7 per cent of the iodine was recovered in the urine in six hours, and 14 per cent in 24 hours.[150] By contrast, when the same amount of crystalline thyroxine was given by mouth, only 1 per cent was excreted in the urine in six hours, and only 6 per cent in 24 hours. Accordingly, the feces alone may yield 54 per cent of the iodine so fed within 24 hours, and 86 per cent in a few days.[493] Likewise, certain dogs given huge doses of crystalline thyroxine (60 mg.) by mouth excreted only from 5 to 9 per cent of the iodine in the urine,[25] whereas, in certain other dogs fed an *alkaline* thyroxine solution, only 9 per cent was excreted by feces. Naturally, if intestinal absorption is poor (and consequently the fecal excretion high), the urinary elimination will be low.

Even after a single large parenteral administration of thyroxine, nevertheless, the urinary iodine elimination is small and markedly delayed,[52] although the extra urinary iodine continues to be detected for several days. A partial explanation of the low fraction of thyroxine iodine eliminated by the kidney is the elimination by the liver and bowel. In the rat, after the intravenous injection of 1.8 mg. of thyroxine, almost 87 per cent of the iodine appears in the feces,[298] but in dogs much less fecal excretion occurs in 14 hours, i.e., 334 gamma of 6500 gamma iodine given as thyroxine.[493]

The mechanism of this fecal excretion is multiple. (*1*) Part of the medication is not absorbed. (*2*) The bile eliminates iodide and probably also some organically bound iodine. (*3*) The large bowel excretes iodide. Thus, Schittenhelm and Eisler found that even the partially isolated large bowel responded to the intravenous injection of thyroxine. Within two hours the iodine in bowel contents had increased from 3 to 26 gamma, and in 14 hours to 76 gamma. (*4*) The stomach wall excretes iodine and (*5*) so does the parotid.[326] Probably a complicated combination of excretion and reabsorption takes place in the intact animal.

Iodine Elimination by the Liver

Whereas the simpler iodine compounds are eliminated by the kidney, more complex compounds are eliminated by the liver, which also can destroy these iodine compounds. It is very likely that the natural, endogenous thyroid hormone is similarly decomposed and excreted. The hepatic cells themselves probably eliminate the iodine, because the biliary iodine is not diminished by the blocking of stellate cells, but does decline with injury to hepatic cells from phosphorus or chloroform.[366]

The iodine excreted in the bile varies markedly with the diet and the season. In the fasting animal the level in bile is similar to that of blood, i.e., 4 to 14 gamma per cent.[148] * After the ingestion of iodine-containing fodder, the rabbit's bile may rise to 55 gamma per cent within three hours. Similarly, in cattle the summer biliary iodine excretion may be more than double that in winter, due to greater iodine intake in fodder.[434] In consequence, wide variations in biliary iodine are recorded in the literature, i.e., in cattle from 7 to 26 gamma per cent; [434] in rabbits from 20 to 62 gamma per cent; [374] in dogs from 16 to 115 gamma per cent.[493]

In this way the liver controls the level of iodine compounds in the circulation and, indirectly, in the tissues. Indeed, the biliary concentration responds more markedly to iodine intake than does the blood. Thus, with a rise in blood iodine (after feeding) from 13 to 16 gamma per cent, a rise in biliary iodine occurs from 9 to 45 gamma per cent.[148] At best, relatively little extra iodide is eliminated by the bile even when it is given intravenously. Thus, in 12 hours only 5 per cent of 1300 micrograms iodine injected as potassium iodide are recovered,[148] because, although there is a continuous biliary excretion after the intravenous administration of iodide, it is weak.[298] The biliary iodine, in turn, is reabsorbed in part by the intestine, so that only from 9 to 12 micrograms of iodine are found in the 24-hour feces when the corresponding biliary output is as high as 62 to 162 micrograms daily.[148]

Organic Iodine Compounds in the Liver. Whereas the liver deals with relatively little inorganic iodide, it is peculiarly adapted to handling organic iodine compounds. Following the absorption from the intestine of a substance like tetraiodophenolsulphonphthalein, the iodine compound proceeds by way of the portal vein to the liver. Here it is temporarily retained, a process called "iodopexis" by Franke [183] and quite analogous to homeostatic mechanisms which deal with a wide variety of other substances. After a large dose of "thyroidin," Zawadowsky and Asimoff [584] were unable to find hormone in the blood until some four hours had elapsed, and during this time the liver is known to accumulate hormone. From about 5 to 20 hours after administration, the blood is strongly positive to biological test, largely due to a reflux of hormone into the circulation. Meantime, elimination of iodine into the bile also tends to reduce the liver iodine concentration further, as iodine compounds are progressively concentrated in the gallbladder bile — a fact which Graham [203] applied in cholecystography. It should be noted, of course, that these observations are distinctly unphysiological, but are of considerable pharmacological interest.

---

* This "endogenous" excretion increases in hyperthyroidism, as explained earlier.

The liver handles proportionately more diiodotyrosine than iodide, and even more thyroxine, once these substances have reached the blood.[298] It tends to decompose thyroxine, but is less efficient when large doses are administered. When thyroxine is administered to animals and the bile assayed, definite amounts of hormone can be detected, although less than its iodine content indicates. Probably between one-half and two-thirds of the thyroxine can be eliminated, undecomposed, in the bile, as judged by chemical fractionation.[35, 585] When thyroxine is given by mouth, or directly into the intestine, however, only a few per cent of the iodine appear in the bile, i.e., 1 to 2 per cent,[489] or 7 to 10 per cent at the highest.[25] After the *parenteral* administration of thyroxine, however, widely varying percentages for biliary iodine elimination appear in the literature, e.g., 43 per cent; [273] 47 per cent; [298] 15 to 47 per cent; [32] 5 to 7 per cent.[148] Several factors may conceivably explain these variations; for example, toxic damage to liver parenchyma, or the effect of various anesthetics on iodine in body fluids.[314, 497] The duration of the experimental period during which elimination is followed also influences the results. When thyroxine is administered in peptone combination (as dry thyroid), urinary excretion preponderates over biliary excretion. Thus, after feeding "thyroidin," only 5 or 6 per cent of the iodine is found in the bile, compared with 93 to 95 per cent in the urine.[25] This difference from thyroxine may be due in part to the greater solubility of protein and to the diiodotyrosine present in "thyroidin." [32] Hepatic action also contributes, however, because after a large dose of "thyroidin" the hormone can be detected easily in the liver, but is found to be largely decomposed in the bile.[584]

THYROID DISTURBANCE

Hypothyroidism. After thyroidectomy in dogs the urinary iodine increases to about double for nearly two weeks, whereupon the excretion returns to normal levels.[526] * This is the period in which the endocrine stores of iodine are declining, as explained in Chapter II. In man, the average daily loss for a week after operation is about 601 gamma (ranging from 11 gamma to 2635 gamma); later the level is normal.[114] In the athyreotic organism, a few days' fast suffices to reduce urinary iodine to almost nothing, whereas in normal animals much less decrease occurs. This low endogenous catabolism, however, is highly abnormal because in myxedema several endocrine glands are presumably "sick" and unable to maintain their normally high stores of iodine. On resumption of feeding after a fast, the urinary iodine again increases, but more so in the athyreotic than in the normal organism, whose thyroid and other tissue stores must be replenished.

In clinical cases, urinary iodine is usually within normal limits.[494] Elmer and Scheps [155] found a daily range of from 40 to 42 micrograms; in only one case of several was it very low, i.e., 10 micrograms. The urinary concentration of iodine was likewise normal. Obviously, the amount of exogenous iodine intake is the controlling factor in the excretion of these individuals. The feces show normal values, i.e., 2 to 11 micrograms for 24 hours.[494] A characteristic balance is shown in Fig. 27.

When fasted dogs are given food high in iodine, the normal animal is able to store iodine better than the athyreotic.[526] Under ordinary circumstances the urinary iodine excretion is like the normal, although more erratic. With thyroidectomized goats, however,[106] the feeding of potassium iodide elevates the iodine elimination during the first few days both in milk and in urine, and does so faster and more markedly than in normal goats. In five days, for example, the urine may contain 99 per cent of the iodine, as against 31 per cent in the urine of normal animals. Even after painting the skin with tincture of iodine, the hypothyroid animal may show a more rapid elimination.[168]

---

* In thyroidectomized goats, even the iodine in the colostrum or milk may fall to a trace.[106]

In the clinic, similar observations have been made in cretinism [443] and in human myxedema [139] (p. 451); during the first six hours after administering iodide the difference from the normal is marked. Furthermore, the difference varies with the degree of hypothyreosis. As stated elsewhere, these results depend upon fixed conditions of dosage and time. Despite Schittenhelm and Eisler's [494] finding of a *decreased* elimination, it seems likely that Elmer's results are consistent and useful when the proper conditions are observed. They again support the thesis that the athyreotic organism traps iodine poorly.[137]

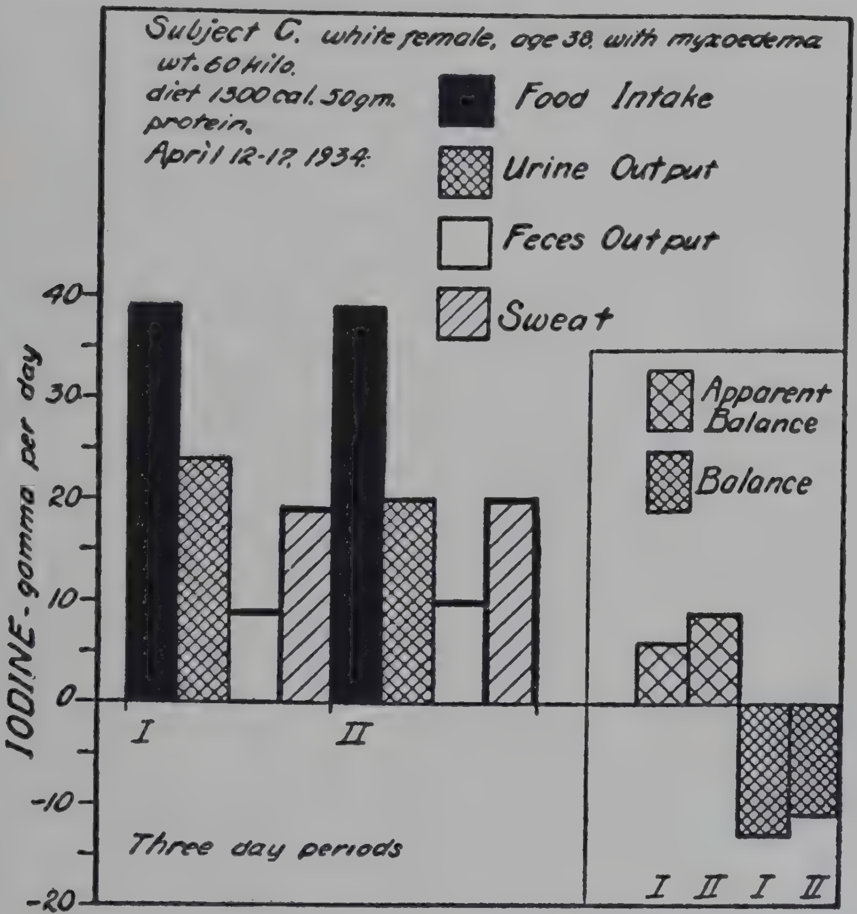


FIG. 27. Iodine balance in a case of myxedema. For comparison with the preceding figure.

From: V. V. Cole and G. M. Curtis, J. Nutrition, *10*: 502. 1935.

When thyroxine is administered to myxedematous men, extra iodine appears in the feces and urine.[493] In myxedema the urinary excretion predominates, whereas in euthyroidism and in thyrotoxicosis the reverse is true. When undissolved thyroxine crystals are fed, only 26 per cent are retained in the body, as against 47 per cent after intravenous therapy; accordingly, the extra urinary iodine, i.e., iodide, excretion is lower. Boothby, Buckley, and Wilhelmj [58] suggest that there is a slower destruction of thyroxine in the hypothyroid organism than in the normal, in view of their finding that the athyreotic dog derives more calorigenic effect from thyroxine than the normal dog.

Hyperthyroidism. In hyperthyroidism one would naturally expect to find an increased excretion of iodine. In mild cases, however, the iodine excretion may be normal, presumably due in part to the retrapping of iodine from destroyed hormone. In Lwów, where the normal urinary iodine averages from 20 to 40 micrograms daily, Elmer found 57 to 133 micrograms in severe hyperthyroidism, and the iodine concentration in the urine may reach 17 gamma per cent. Other observers report similar values for daily excretion, e.g., from 55 to 949 micrograms, averaging 171 micrograms,[115] and from 8 to 290 micrograms.[482] Representative values are given in Table 39. Elmer and Scheps [154] studied the increased urinary iodine by chemical analysis and were unable to find thyroxine-like material present. Apparently the hormone is decomposed before excretion.

When urinary iodine is high, the blood level and metabolic rate are usually also elevated, though not necessarily in proportion. During preoperative excitement there may be very high urinary excretion (e.g., 949 micrograms of iodine).[114] Likewise, the onset of menstruation in the hyperthyroid woman may induce a slight increase. Elimination by other paths may also be increased. Doubtless sweating by the moist, flushed skin in Graves' disease plays an important role in diverting the outgoing stream of iodine. Occasional cases of human hyperthyroidism show a normal iodine excretion in the urine [96] because of increased loss by sweat and feces, and not infrequently patients show more than a normal amount of iodine in the sweat.[482] Likewise, Scheffer found increased iodine in the expired air, amounting to (roughly) 14 to 26 micrograms.

Biliary elimination of endogenous iodine increases also. For example, in a case of moderate hyperthyroidism with a blood iodine of

TABLE 39

THE IODINE BALANCE IN DISEASES OF THE THYROID GLAND; A COMPARISON OF THE IODINE BALANCE OF NORMAL INDIVIDUALS WITH THAT OF NODULAR AND EXOPHTHALMIC GOITER PATIENTS

Type of goiter present	No. of pts.	Total days of investigation	Av. BMR	Av. blood iod.	Av. Output/3-Day Period				Av. intake per 3-day period	Av. balance per 3-day period
					Urine	Feces	Sweat	Total		
			%	γ %	γ	γ	γ	γ	γ	γ
1. None: normal controls . .	3	24	−7	4.3	154	31	28	213	87	−126
2. Non-toxic nodular	2	18	−8	3.0	120	31	29	180	74	−106
3. Toxic nodular	2	15	+28	8.5	323	149	38	510	117	−393
4. Exophthalmic	3	33	+40	9.0	204	164	46	414	86	−328

From: I. D. Puppel and G. M. Curtis, J. Clin. Investigation, *17*: 736. 1938.

23 micrograms per cent and a daily urinary excretion of 87 micrograms [139] (p. 383), the bile showed a concentration of 18 to 19 micrograms per cent. Even though partial reabsorption in the intestine would tend to lessen this effect, as much as 43 to 237 micrograms of iodine daily may be excreted in feces.[96] Scheffer's [482] values were 7 to 118 micrograms. Of course, the fecal excretion varies inversely with excretion in urine or sweat.

In summary, then, there is a tendency to negative iodine balance in hyperthyroidism. In mild cases it may be imperceptible, but in severe cases it may exceed 200 micrograms of iodine daily. This depletion of iodine occurs by the combined effect of several routes of excretion.[534] In non-toxic nodular goiter, as shown in Table 40, the iodine excretion is normal; whereas in toxic nodular goiter excessive catabolism leads to depletion of the iodine reserves. Elmer points out that in severe cases the reserve of iodine in thyroid would be depleted in from three to eight months, unless extra iodine were supplied. The gland presumably retraps part of the iodine from decomposed hormone and uses it again for fresh hormone. Even so, Perkin and Hurxthal [431] have reported cases of neglected severe "thyrotoxicosis" with normal blood iodine levels and only moderately increased basal metabolic rate, despite clinical evidence of marked Graves' disease. Studies of iodine balance in such cases would be extremely interesting.

Immediately after surgical treatment of hyperthyroidism, an excessive elimination of urinary iodine occurs. Presumably, part of the extra iodine excretion after thyroid surgery comes from manipulation of the thyroid,[525] but much of it may come from extrathyroidal tissues. Indeed, human patients after undergoing simple thoracoplasties may lose 2515 to 3109 micrograms of iodine in the first day after operation, a loss even greater than that after thyroidectomy.[114] After roentgen therapy delivered over the thyroid in a single case, Elmer [139] (p. 416) found that a fall in urinary output occurred as the hyperiodemia and basal metabolic rate decreased. More data are needed.

Rowe [464] pointed out that hepatic disturbance was more frequent in Graves' disease than in any other endocrine disease. Indeed, a wide variety of liver lesions is found in hyperthyroidism,[42] and such disturbance is regarded by some clinicians as a characteristic feature of the disease. Perhaps this phenomenon is partly explicable on the

TABLE 40

Iodine Balance in Nodular Goiter, Nontoxic and Toxic

Period	Date begun	Wt.	Iodine						Date	Blood iodine	B.M.R.	Remarks
			Output									
			Urine	Feces	Sweat	Total	Intake	Balance				
Nontoxic *	1937	kg.	γ	γ	γ	γ	γ	γ	1937	γ %	%	
I	March 1	87	63	29	31	123	92	−31	March 3	2.2	−10	Bilateral subtotal thyroidectomy, March 8, 1937.
II	March 4	87	91	27	32	150	57	−93	March 6	2.2	−8	
III	March 7	87	2334	26	161	2521	75	−2446	March 8	2.2	−10	
IV	March 10	87	268	46	30	344	59	−285	March 10	3.1	−5	
									March 12	2.2	+12	
V	March 13	87	191	34	30	255	75	−180	March 15	1.9	+7	
Toxic †	1938	kg.	γ	γ	γ	γ	γ	γ	1938	γ %	%	
I	Jan. 28	49	303	333	30	666	120	−546	Jan. 27	9.5	+27	General hospital management.
II	Jan. 31	50	298	145	23	466	121	−345	Jan. 30	8.5	+19	
III	Feb. 3	50	293	120	44	457	120	−331	Feb. 2	8.5	+35	
IV	Feb. 6	50	326	101	39	466	122	−344	Feb. 5	9.0	+24	
V	Feb. 9	..	2793	185	405	3383	120	−3263	Feb. 7	8.1	....	
VI	Feb. 12	..	513	138	27	678	120	−558	Feb. 10	8.1	....	Bilateral subtotal thyroidectomy, Feb. 9, 1938.
XI	Feb. 27	48	302	32	16	350	110	−240	Mar. 1	2.0	−10	
XII	Mar. 2	48	231	29	22	282	113	−169	Mar. 4	2.4	−10	

* B.S., No. 370995, female, age 35; preoperative diet 2250 calories, 52 grams protein; nontoxic nodular goiter.
† R.J., No. 380303, female, age 31; toxic nodular goiter; diet 2600 calories, 64 grams protein.
From: I. D. Puppel and G. M. Curtis, J. Clin. Investigation, *17*: 731–732. 1938.

basis of the increased amount of hormone with which the liver deals. As explained elsewhere, this functional strain is apparently so great that the liver fails to destroy all of the hormone before eliminating it in the bile.[32] Moreover, the histological condition can be simulated by giving excessive doses of thyroxine or thyroid to normal animals.[128]

Simple Goiter. The urinary iodine excretion tends to be low in goitrous districts.[168] Fecal iodine, however, tends to be approximately normal, i.e., from 5 to 14 gamma per cent (normal values are 3 to 5 gamma iodine).[484]

The goitrous individual traps iodine more efficiently than the normal.[241] Thus, when large doses of inorganic iodide are ingested by patients with simple goiter, they eliminate only about one-fourth of it in the urine in two days, whereas normal controls excrete one-half.[483, 218] Similarly, with a small intravenous dose and a shorter time interval, Elmer found 3 to 19 per cent elimination in goiter (nine cases), whereas the extreme low limit of normal is 12 per cent.

CHAPTER X

RADIOACTIVE IODINE

DURING the past decade great advances have been made in biochemical research through the use of "tagged" atoms. Some of the most effective pioneer work of this sort was done by Schönheimer [501] with heavy hydrogen and heavy nitrogen. About the same time Hevesy [243] and his collaborators, and August Krogh,[300] initiated their studies with radioactive phosphorus; and, with the development by Curie, Joliot, Fermi, E. O. Lawrence,[308] and others of the field of radioactive isotopes, this latter method of tagging substances has proved increasingly valuable. In 1938 Hertz, Roberts, and Evans [240] reported physiological studies with radio-iodine which were undertaken in 1937, but at the time these experiments were begun the usefulness of the method was limited by the fact that the half-life of the sole isotope then available was only 25 minutes. In consequence, as was pointed out also by Hamilton,[217] who began investigations in 1938, studies making use of this short-lived material could not profitably be pursued for a period much longer than two hours. Meantime, in 1938, the possibilities were greatly extended by the discovery of three other radioactive isotopes of iodine by Livingood and Seaborg,[329] and by Tape and Cork,[535] so that now observations extending over a period of several weeks are feasible. Thus far the isotope which has been used most is that with an atomic weight of 131, because it has a half-life of eight days and can most readily be prepared with high intensities of radioactivity.

Such tagged radioactive elements behave chemically exactly like the natural elements, as pointed out by Goudsmit.[200] Consequently, in their history within the animal organism, they suffer the same fate as their fellow atoms which are not radioactive.[219] Usually, only one such atom exists among many hundred thousands of its non-radioactive fellows, but methods for detecting the energy given off by this lone atom are now so refined that the concentration of the radioactive material is negligible in comparison with concentrations of the inactive material which is participating in metabolic processes. At irregular intervals some of the radioactive atoms distintegrate and, in consequence, the concentration of radioactive material gradually diminishes. In the case of I^{131}, half of the initial material has disappeared at the end of eight days, and in the next eight days half of this remainder disappears. Thus the "half-life" of this isotope is eight days, and I^{131} is referred to as "the eight-day isotope." By using an inverse logarithmic curve, the investigator is able to control the residual concentration of radioactive material throughout the course of his experiment. Eventually, however, so few radioactive atoms remain that the Geiger-Müller counter is no longer able to measure them accurately.

After such material has been calibrated for its initial activity, it may be administered to a man or animal; and subsequently, measurements may be made of its distribution. If the total concentration is sufficiently high, the total activity of the organism may be measured directly, or certain portions of the organism may be measured *in vivo*, such as the thyroid of a goitrous patient. Alternatively, the thyroid or other tissues may be removed from an experimental animal, or from a patient at operation, and extracts or pastes ("brei"), may be subjected to simultaneous analyses for total iodine and for radioactive iodine. Likewise, the blood and excreta of the animal or patient may be collected, and metabolic balances obtained in terms of radioactive material.

A considerable number of technical considerations lie at the basis of such investigations, and it will be possible to suggest only a few of them here in brief form. First of these is the fact that mixtures of isotopes may occur. For example, the eight-day radio-iodine often is accompanied by small amounts of four-day and 13-day iodine. In order to evaluate properly the natural history of the radioactive material in the animal at successive time intervals, it is necessary to measure the composite decay curve of such a mixture of isotopes. More serious is the possible presence of nuclear isomerism which may be present when the nucleus of a radioactive isotope exists in two states. Each of these will have a different half-life and one isomer may exist in a highly unstable form for an appreciable period of time (the "metastable" state). Thus, two different types of isomerism may occur. In one type each of the two isomers decays by the emission of a beta-ray, independently of the other. More commonly, another type of decay occurs; this process produces a complicated sequence of events involving the ejection of an electron and the breaking of any chemical linkage in which the atom may have participated. In order to rule out this complication, which might entail a serious error in metabolic studies, Hamilton and Soley [218] examined the available radio-iodines for this second form of decay. Fortunately, they were able to eliminate its presence in I^{128} (25-minute half-life) and in I^{131} (8-day half-life), thus putting on a sound basis the use of radio-iodine as a metabolic procedure.

Radioactive Iodines

At least five radioactive iodines have been described recently by Livingood and Seaborg,[329] three of which were newly discovered. They were produced by the bombardment of iodine, tellurium, or antimony by neutrons, deuterons, or helium ions, respectively. These various isotopes have been summarized by Roberts, Evans, and Hertz [461] in Table 41. Of course, naturally occurring iodine is I^{127}.

TABLE 41

Isotopes of Iodine

Isotope	Half-life
I^{124}	4.0 ± 0.3 days
I^{126}	13.0 ± 0.3 days
I^{127}	stable
I^{128}	25.5 ± 1.0 minutes
I^{130}	12.6 ± 1.0 hours
I^{131}	8.0 ± 0.2 days
I^{132}?	2.4 hours
I^{134}?	54 minutes
I^{136}?	22 hours

A convenient preparative method, using the cyclotron, is the bombardment of 1 to 2 grams of metallic tellurium with deuterons. The major part of the radioactive material thus produced consists of the eight-day radio-iodine, I^{131}; although four other radioactive isotopes are produced simultaneously, either they are short-lived or present in such low concentrations as to be negligible, as reported by Hamilton and Soley [218] under certain conditions of bombardment. The treated sample of tellurium can then be dissolved in nitric acid and distilled together with added inert iodine, which carries over with it the radio-iodine. The resulting product is collected as a solution in carbon tetrachloride, from which it may be extracted with aqueous sodium thiosulfate. In this way it is possible to obtain a known standard solution of "labeled" iodine. The amounts of the original radioactive material are of the order of 10 to 50 micromicrograms mixed with an equal number of milligrams of inert iodine, at best. As usually adulterated with inert iodine, the radioactive concentration is far less.

It is beyond the scope of this chapter to discuss in detail the properties of these various isotopes, but because the 8-day radioiodine, I^{131}, will doubtless prove to be an important physiological and clinical tool, a brief sketch of its properties may be given. Livingood and Seaborg[329] suggested that a double decay occurs from tellurium to iodine to stable xenon, and that the sequence of events might be either Te^{129} to I^{129} to Xe^{129}, or else Te^{131} to I^{131} to Xe^{131}. Very recently Abelson[11] has described iodine with period 131 as a fragment of uranium disintegration; and Seaborg, Livingood, and Kennedy[502] report evidence favoring I^{131} as the precise status of the eight-day isotope. For the moment, however, this distinction appears to be of no importance in physiological procedures under the conditions just outlined. In either case, the decay curve can be plotted as a linear semi-logarithmic function of time.

Nevertheless, as pointed out by Hertz, Roberts, Means, and Evans,[241] for special purposes the other radio-iodines may prove very useful. For example, successive administrations of eight-day isotope I^{131} and of twenty-five-minute isotope I^{128} may be used to distinguish the initial progress of iodine metabolism as compared with its final outcome. In other words, it is possible in this way to distinguish the fate of the first dose of iodine from that of the later dose. At the present time, however, due to the relatively slow turnover of iodine in physiological processes, the I^{131} has proved most useful, and most of the physiological data now available in man have been obtained with this isotope. Perfectly adequate and highly illuminating observations, however, can be made in animals with the short-lived I^{128}, as will be shown.

"Labeled" Iodine. This term is used to designate ordinary iodine (or a chemical compound thereof) which is distinguished by the admixture of a small amount of radio-iodine existing in the same type of chemical combination. Thus far, biological work has been limited largely to inorganic iodide. The "labeled" iodine, therefore, is merely ordinary iodine in which one atom of (perhaps) every several million is "tagged" by its radioactivity. Because these "tagged"

atoms behave chemically precisely as do their fellows, the investigator may assume safely that, wherever he can detect one of these (perhaps) several million comrade atoms are present. Of course, the "tagged" particles make known their presence by blowing up; consequently, their relative number with respect to their comrades diminishes exponentially with time.

During a biological experiment the observer must know this ratio of radioactive to inert atoms at any given moment so that he may calculate the total amount of iodine betrayed by the presence of the "tagged" atoms. At first thought, this would seem to be difficult, because the ratio is constantly changing as more and more radioactive atoms disappear. In practice, the problem is readily solved. Whenever the biologist performs an experiment, he saves out a small portion of the material as a standard sample. If all subsequent measurements are referred to this sample, this standard automatically controls the atomic decay occurring in the main mass of material used.

Application of the Method. Once a preparation of iodide containing a known amount of radioactive element has been obtained, it may be used for administration to man or animals under suitable conditions. Hamilton and Soley[218] have given doses of 14 mg. of "labeled" iodide to human subjects by mouth in the morning before breakfast. It was found that over 80 per cent of the iodide so administered was absorbed through the digestive tract at the end of one hour. Just before administration, the ionization of the beta-rays from the radioactive material was used to determine the initial activity of the preparation. For this purpose a Lauritsen quartz fiber electroscope could be used for the estimation. The time was thus noted, and thereafter, allowance could be made for the regular decay of the material involved. The urine was collected in twenty-four-hour samples and the feces collected during a five-day period, during which observations were made. When thyroidectomy was performed on such a patient, preferably two to four days after the administration of the radio-iodine, the amount of thyroid tissue left in the patient was estimated carefully; because this quantity was always a small fraction of the portion removed, no significant error in the total weight of the gland was introduced.

The urine, the feces and the tissue removed had to be prepared for the accurate determination of the radio-iodine content by special procedures which are complicated as follows. First, there is the complication that the preparation of radio-iodine samples of adequate activity requires prolonged bombardment of the tellurium by the cyclotron. Secondly, the gamma-ray emission of the 8-day radio-iodine is very weak, and, thirdly, the beta-rays resulting from the breakdown of this material are so soft that one millimeter of tissue will screen out over 90 per cent of them. In consequence it was necessary to find a procedure which would permit quantitative extraction of the radio-iodine from large amounts of biological material. Finally, it was decided to treat the material very much as in the analytical methods of Leipert,[312] Trevorrow and Fashena,[550] or Matthews, Curtis, and Brode,[369] as described in the appendix of this book. The iodine contained in the extract of the tissues was re-distilled with a known amount of added inert iodine as an aid and a check on the recovery of "labeled" iodine. This procedure was finally perfected so that about 97 or 98 per cent of all the material could be recovered from urine, feces, or thyroid gland.

Final Measurement of Radio-Iodine. Hamilton and Soley [218] used a vacuum tube electrometer to detect I^{131}, as described by DuBridge.[129] This instrument they considered more satisfactory than the Geiger-Müller counter when soft beta-rays are to be measured. With this technique it was possible to measure a radioactivity equivalent to 5×10^{-9} grams of radium within an error of 5 per cent. Nevertheless, Geiger-Müller counters *have* been used satisfactorily for the detection of the I^{131}, as shown by Roberts,[460] with an accuracy of 5 per cent for an equivalent of 5×10^{-11} grams of radium. No correction for decay of the samples was necessary because standards were measured at the same time by the same apparatus, always within an hour.

Preparation and Measurement of Short-lived Radio Iodine. Although there are many advantages to be had with the eight-day isotope, I^{131}, there are a number of interesting observations possible with the short-lived isotope, I^{128}, having a half-life of about 26 minutes. Such material was described originally by Hertz, Roberts, and Evans.[240] This material was prepared from ethyl iodide, which was irradiated in a paraffin-surrounded bottle by immersing in it a source of neutrons consisting of radium mixed with beryllium in a sealed tube. This radioactive material was then concentrated by a method of Roberts and Irvine as adapted from d'Agostino [12] as follows. A few milligrams of free iodine (about 1 to 100) were added to the irradiated ethyl iodide, and an equivalent weight of sodium bisulfite in water was shaken with the ethyl iodide to extract it. The aqueous layer was separated, and the iodide then precipitated by adding an excess of slightly acid silver nitrate solution. In this way almost all the activity could be concentrated in the precipitate of silver iodide. Before intravenous injection, this material was dissolved in aqueous sodium thiosulfate. When prepared in this way, it was tolerated well by rabbits. The ethyl iodide could be used over and over again. The complete separation required less than ten minutes.

This method is recommended by Roberts and Irvine.[462] These last authors failed to discover any gamma-rays emitted by this material, although beta-rays are characteristic. The material can be analyzed by means of a Geiger-Müller counter attached to a suitable vacuum tube amplifier, which records the disintegration of the individual radioactive atoms. It was necessary for the experimenters to work rather rapidly because accurate observations could not be extended beyond forty minutes after the beginning of the experiment. With more intensely radioactive material this time can be extended conveniently up to several hours. The tissues involved were excised, then finely minced and spread on a flat surface which was placed in direct contact with a Geiger-Müller counter. By this method a number of tissues were examined, i.e., thyroid, ovaries, hypothalamus, cerebral lobe, pituitary, salivary glands, liver, spleen, and muscle. It was possible also to analyze body fluids, such as blood, urine, and cerebrospinal fluid. These acute observations are of considerable interest because they demonstrate the extreme specificity of the thyroid in trapping iodine. This biological selection did not apply to "labeled" bromine, surprisingly enough. These experiments showed also that when the gland was hyperplastic, the ability of the thyroid to trap iodine in a short time was increased severalfold, and this accumulation occurred within fifteen minutes after injection. Furthermore, studies of the urinary excretion for half an hour following injection indicated that rapid and prompt elimination of the radio-iodine occurred and, as was to be anticipated, followed a semi-logarithmic law.

Observations with Short-lived Radio Iodine

Hertz, Roberts, Means, and Evans [241] found that the normal rabbit thyroid could collect up to eighty times the quantity to be expected from uniform diffusion throughout the body tissues. In hyperplastic thyroids, indeed, this relative concentration could reach several hundredfold. After the intravenous injection of radio-iodine, the percentage of any given dose to be collected was found to reach a maximum within ten to fifteen minutes, and periods as long as an hour and a half did not increase greatly the amount trapped. These authors, furthermore, used several different types of tagged iodine, as suggested earlier in this chapter, i.e., combinations of short-lived atoms with long-lived atoms. Most of their observations were made with the radioactive isotope with mass 128, which has a half-life of twenty-five minutes; but they used also radioactive iodine with the half-periods of 12.5 hours, eight days and thirteen days, respectively. These correspond to the respective mass numbers 130, 131, and 126. In this way, the investigators were able to extend observations over a wide range of time: namely, in acute experiments for only one and one-half hours, but in chronic experiments up to eight days. In order to standardize the relative weight of the thyroid, the authors used a so-called "thyroid index," which was calculated by dividing the weight of the thyroid in milligrams by the body weight in kilograms multiplied by 10. This index in most animals ranged between 5.5 and 8.0. When the gland was hyperplastic, however, the values ranged from 8.0 to 33.5. This last value was obtained in an animal which had been on an exclusive cabbage diet for two months.

In order to correct for the variation in size of thyroid glands, the authors used also a "thyroid concentration coefficient," which was calculated by dividing the thyroid index into the thyroid iodine collection in per cent, and multiplying the whole by 1,000. Thus the "thyroid concentration coefficient" equals the concentration of iodine in the thyroid divided by the average concentration of iodine in the body. In other words, it expresses the concentrating power of the thyroid relative to the concentration to be expected from uniform diffusion throughout the rest of the body tissues. These investigators found that the concentration of iodine in the thyroid increased until the total collection had reached about 40 to 50 micrograms; after that, no further trapping of iodine occurred. The time necessary to reach this maximal concentration was as short as fifteen minutes and this quantity might not increase much even after eight days. Thus there seems to be a "ceiling" in normal thyroids beyond which no further concentration of iodine occurs.

Apparently it is also very easy to saturate a hyperplastic thyroid, if a dose of correct magnitude be given. In this latter sort of gland many factors appear to affect the ability of the thyroid to accumulate iodine, such as the quantity of the previous dose and the time which elapses after it, the type of thyroid stimulation, and the "thyroid index" of the animal. In both normal and hyperplastic glands, indeed, there is an initial collection which is maximal by the end of ten minutes and does not increase for at least one and one-half hours. This time is as short as one would anticipate from the circulation rate, and the rate at which the thyroid traps this initial collection of iodine is phenomenal as compared with the rate at which it accumulates iodine subsequently over the course of several days. Indeed it was possible to detect iodine in the thyroid of animals which died within two minutes after injection of the radioactive material. In Fig. 28, taken from Hertz, Roberts, Means, and Evans,[241] is illustrated the striking effect which the functional state of the thyroid has upon its ability to withdraw iodine from the general circulation. From the figure it is evident, first, that within 10 to 15 minutes the initial accumulation has ceased, and secondly, that the amount accumulated depends upon the degree of hyperplasia.

Threshold for Accumulation of Iodine. Of course the dose of iodine given influences the percentage which is trapped. In normal glands, the percentage collected increases as the dose *decreases*. In other words, the gland operates more efficiently when less iodine is supplied, as one might expect from general biological considerations. In more precise terms, the "thyroid concentration coefficient" is about 80 for normal glands when a dose of 0.1 mg. is given, whereas it may be as low as 2 for an injection of 100 mg. Hertz, Roberts, Means, and Evans [241] distinguish two types of hyperplastic gland as contrasted with the normal, namely, Type 1, in which the "thyroid concentration coefficient" is always greater than normal, and Type 2, in which the "thyroid concentration coefficient" may be lower than normal. In the latter type the authors have described a so-called threshold phenomenon, in that injections of less than 10 mg. of iodine are less efficiently accumulated in the gland, whereas injections greater than 10 mg. of iodine are trapped quite efficiently, just as normal and Type 1 animals trap it.

Indeed, in hyperplastic glands there seems to be a continuous gradation in the concentrating power of the gland, particularly with reference to this threshold effect. These observations are similar to observations which are well known to clinicians who treat patients with Graves' disease. In that condition it is clear that small doses of iodine do not particularly affect the basal metabolic rate; whereas, large doses of iodine have a striking effect in stopping thyroid secretion into the circulation, while at the same time enabling the gland to accumulate surprisingly large amounts of iodine to be stored as thyroid colloid. Furthermore, clinicians also recognize that in the early phase of colloid goiter, when hyperplasia is present, the administration of iodine has no effect upon gross thyroid function; and yet large amounts of iodine are stored in the gland.

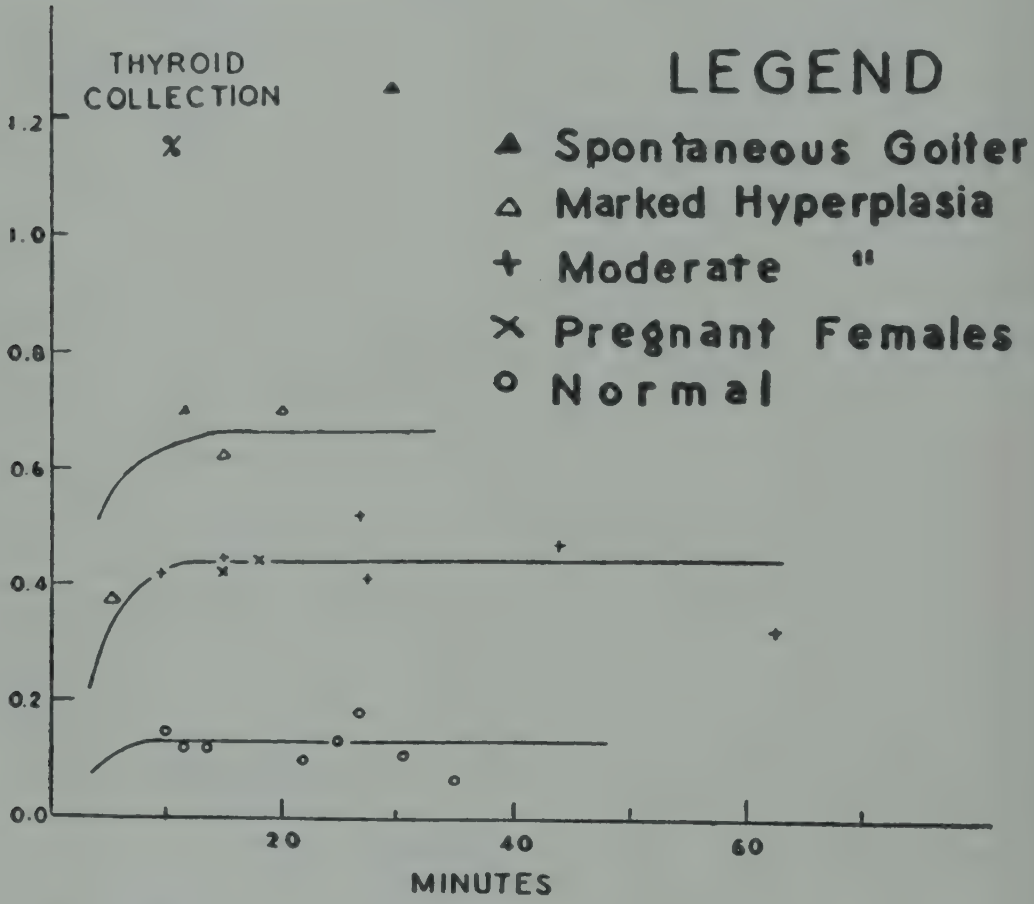


FIG. 28. When 10 mg. of iodine (as iodide) were injected into rabbits, the trapping of this additional iodine was nearly complete in fifteen minutes. The efficiency of the collection varied with the functional state of the gland, as indicated.

From: S. Hertz, A. Roberts, J. H. Means and R. D. Evans, Am. J. Physiol. *128*: 567. 1940.

In Fig. 29, from Hertz, Roberts, Means, and Evans,[241] are shown the three types of gland, i.e., normal, Type 1, and Type 2, plotted in rough fashion with respect to their thyroid collection for the same relative mass of thyroid tissue. Type 1 and Type 2 are clearly different from the normal, but, as stated earlier in this chapter, these two types probably merge one into the other. It is quite evident that the hyperplastic gland collects more iodine than does the normal when large doses of iodine are administered; but it seems clear that when small doses are administered some of these hyperplastic glands are less efficient than the normal gland.

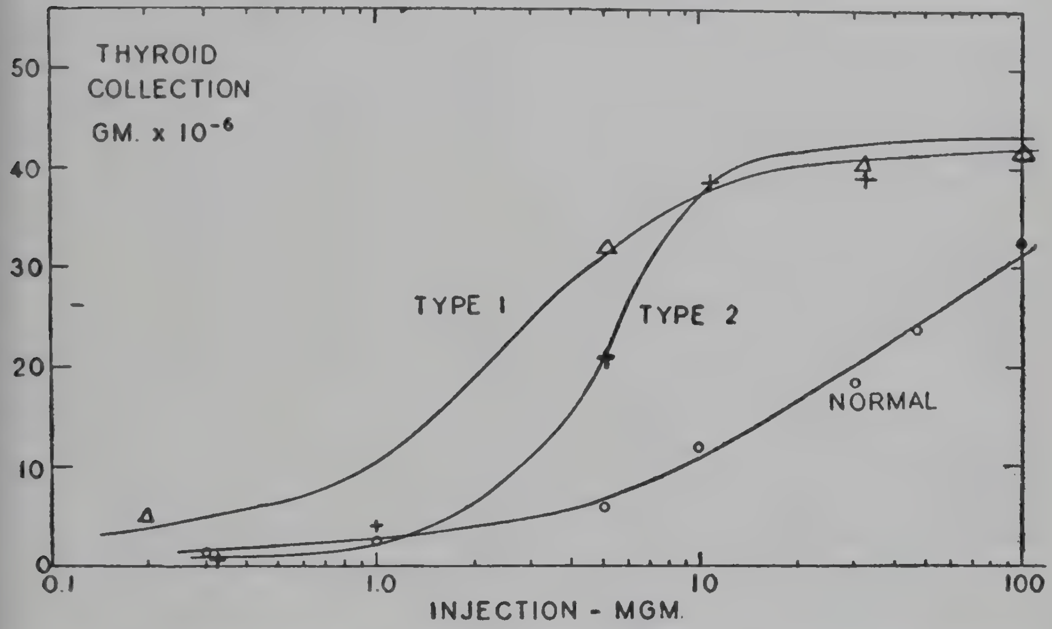


FIG. 29. When rabbits were injected with varying doses of iodide, the total collection in their respective thyroids varied with the functional state of the glands. Three general functional states were found, i.e., Normal, Type 1, and Type 2, as described in the text.

From: S. Hertz, A. Roberts, J. H. Means and R. D. Evans, Am. J. Physiol. *128*: 571. 1940.

This distinction between normal and hyperplastic glands holds for all hyperplasia, so far as is known. In other words, the result will be the same whether the hyperplasia be induced spontaneously, by the administration of thyrotropic hormone, by administration of goitrogenic substances (such as a prolonged cabbage diet), or by the administration of methyl cyanide. It will be remembered that Marine and Rosen[362] used such reagents in order to induce goiter experimentally in rabbits on the supposition that, by so doing, they were producing a relative lack of hormone and consequently a relative lack of iodine. Indeed, Marine [355] has hypothecated, as described in Chapter VI, that all hyperplasia of this sort depends upon the action of thyrotropic hormone; and, so far as these experiments with radioactive iodine are valid, it would indeed seem to be true that all hyperplastic glands had much in common as regards their behavior towards iodine. There remains to be explained, however, the extreme divergence between the characteristic Type 1 and the characteristic Type 2 gland, as well as the apparent threshold phenomenon already described. So far as can be seen at present, it would appear that hyperplasia in the thyroid involves at least two factors, one of which determines total capacity for trapping iodine, and the other which determines the intensity or concentration of iodine necessary to induce such accumulation. Inasmuch as capacity and intensity factors are recognized throughout chemistry and biology, perhaps it is not surprising that they should be encountered in thyroid physiology. Moreover, the intensity factor resolves itself into concentration of blood iodine and this, as described in Chapter V, is a crucial factor in limiting thyroidal activity.

Observations with Multiple Labeling. As regards the amount of iodine which may be absorbed, it is clear that if a thyroid has previously been saturated with iodide a second injection will not yield much further increase. For example, an animal which in 19 hours accumulated 53 micrograms of iodine, trapped less than 4 micrograms from a subsequent injection. By contrast, an animal which trapped only 20 micrograms after 31 hours proceeded to collect 7 micrograms from a subsequent injection. In Fig. 30, from Hertz, Roberts, Means, and Evans,[241] is illustrated the effect of preliminary iodine medication upon the response to a second injection of 5 mg. of labeled iodine. It is clear that both for normal and so-called Type 1 hyperplastic glands the preliminary administration of iodine decreases the avidity of the gland for more iodine. Nevertheless, the normal gland is less affected than the Type 1 gland.

Accordingly, if an animal has been treated with iodine previously, he will collect less iodine subsequently. More precisely, a normal animal after receiving a dose of about 50 mg. of iodine is able on the second injection to accumulate only half of the labeled iodine which the otherwise normal, untreated animal would trap. In the so-called Type 1 animal, as little as 8 mg. of iodine will have the same effect within six hours.

These observations raise several interesting problems which have appeared as by-products. Among these may be mentioned the fact that treatment with one type of thyrotropic hormone appeared to produce a different iodine collection at the same thyroid index than did injections of several other thyrotropic hormone preparations, including the saline suspensions of fresh beef anterior pituitary. It may be that there are separate factors which control, respectively, the functional stimulation and the hyperplasia of the thyroid follicle. Indeed, it is well known to clinicians that the size of the thyroid alone does not indicate functional activity. Another problem raised

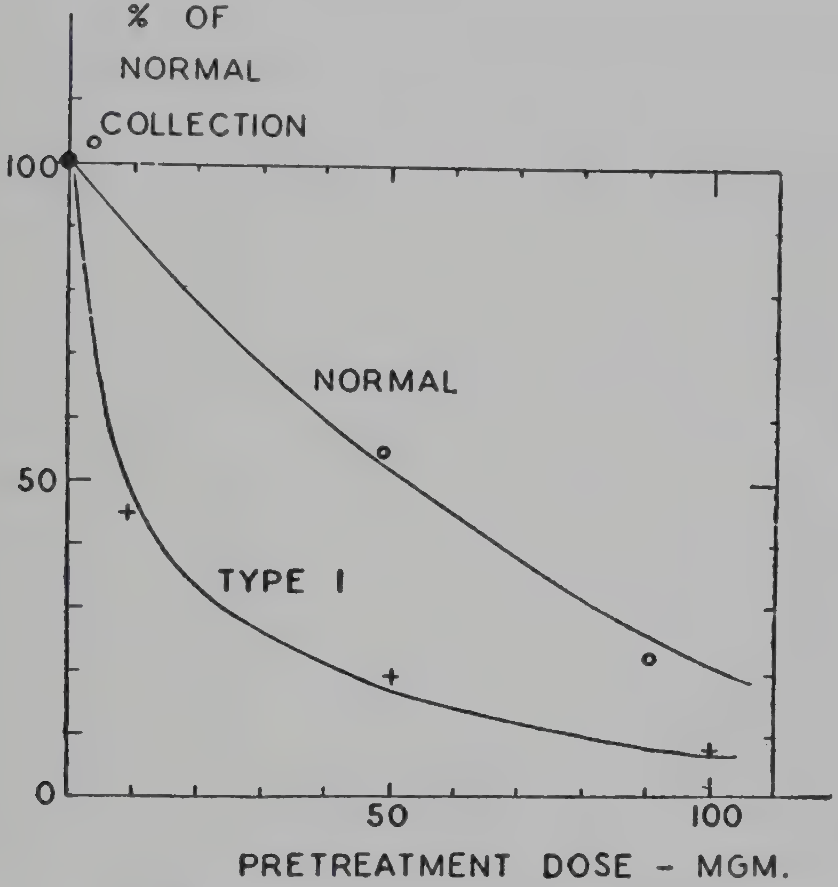


FIG. 30. When rabbits were injected with iodide containing 5 mg. of labeled iodine, the trapping of this dose was impaired if previous iodine had been given. The percentage of the normal collection which resulted depended upon the size of the previous dose. The curve shown for Type 1 animals was for a thyroid index of 10 and for a time-interval (between doses) of four to six hours. The number of animals represented in the normal group was nine; in the Type 1 group, twelve.

From: S. Hertz, A. Roberts, J. H. Means and R. D. Evans, Am. J. Physiol. *128*: 571. 1940.

by these observations was a difference in the behavior of animals whose glands became hyperplastic as the result of a prolonged cabbage diet, as compared with the animals injected with methylcyanide. If the goitrogenic substance in the cabbage diet is a cyanide, as has generally been supposed hitherto, it is difficult to understand this observation.

TABLE 42

RADIOACTIVE IODINE STUDIES IN NORMAL SUBJECTS AND IN PATIENTS WITH SPONTANEOUS MYXEDEMA, AND VARIOUS TYPES OF THYROID DISEASE

	Normal subjects §						Spontaneous myxedema §	
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Urine 1st day	81.0	65.0	75.2	53.1	72.0	52.9	49.8	43.4
Urine 2nd day	5.94	10.85	12.0	19.6	13.0	17.4	26.2	33.4
Urine 3rd day	0.53	1.96	1.18	3.00	0.53	2.36	9.36	9.50
Urine 4th day	0.18	0.63	0.05	0.24	0.06	0.79	4.21	2.60
Urine 5th day	0.48	0.26	0.07	0.30	0.01	0.40	1.84	0.20*
5-day feces	0.98	2.82	0.29	1.62	0.48	0.42	2.63	2.03
Total	89.11	81.52	88.79	77.86	86.08	74.27	94.04	91.13

Thyroid disease ¶	Toxic nodular goiter without Lugol's	Non-toxic nodular goiter without Lugol's		Toxic diffuse goiter, Lugolized			Toxic nodular goiter, Lugolized	Non-toxic diffuse goiter, involuted. (Patient also had carotid sinus syndrome.)
Urine 1st day	52.7	38.3	74.2	64.7	67.7	50.3	73.9	64.3
Urine 2nd day	29.3	11.6	3.90	10.3	19.6	20.0	12.5	3.42
Urine 3rd day	2.40	1.16	0.94	1.67	0.67	..	1.18	0.22
Urine 4th day	0.02†	0.21	0.18	0.37	0.26	..	0.09	0.20
Urine 5th day	0.17	0.20	0.17	0.26	0.08	..	0.03	0.18
5-day feces	1.36	0.08	0.16	2.09	0.06	0.15‡	0.38	11.25
Thyroid	5.50	17.5	12.5	1.07	7.50	7.12	4.32	0.07
Total	91.45	69.05	92.05	80.46	95.87	77.47	92.40	79.64
Total thyroid iodine (mgm)	5.08	3.06	20.8	...	57.3	82.5	62.3	...
Weight of thyroid (grams)	69.4	54.85	87.4	23.2	39.7	71.4	81.1	10.2

§ Radioactive iodine given orally. Excretion in urine and feces during first five days after administration (total iodine given was 14 mgm. as a single dose).

* Patient had a severe gastro-intestinal upset during the 5th day which was associated with a marked oliguria.

¶ Per cent of orally administered radioactive iodine excreted in urine and feces over a 5-day period, and of radioactive iodine taken up by thyroid in 48 hours (total iodine given was 14 mgm. as a single dose).

† Part of sample lost.

‡ 2-day feces.

After: J. G. Hamilton and M. H. Soley, Am. J. Physiol. *127*: 565 and 566. 1939.

EXPERIMENTAL RESULTS WITH I^{131} IN MAN

It is interesting that Hamilton and Soley were able to recover in the urine and feces, within five days, about 80 per cent of the iodine administered to normal subjects. Indeed, in spontaneous myxedema they were able to recover over 90 per cent. As shown in Table 42, the major portion of this recovery was urinary, and the first day's excretion for normal subjects was by far the most significant. In human myxedema, however, there was a definite delay in urinary excretion so that the second day played a significant role in the total excretion. As might have been expected from studies reported in another chapter, fecal excretion at best accounted for less than 3 per cent and in most cases only about 1 per cent. This general technique was then applied to a number of patients with various types of thyroid disease. These studies are admittedly incomplete, but several interesting points have already been unearthed. For example, when patients with different types of goiter, namely, toxic goiter and nontoxic nodular goiter, were studied and compared with normal controls, the rate of excretion in all cases was found to be much the same. It will be recalled, however, that Curtis[96] found a more rapid excretion in patients with toxic goiter who received rather large doses of iodide. By contrast, the rate of excretion in the urine of myxedema patients showed a much slower eliminative period. As would be expected, all cases showed a linear excretion when the per cent of iodine excreted in the urine was plotted against time, as shown in the accompanying Fig. 31. This phenomenon simply indicates that a rather constant proportion of the iodine in the body is excreted each day, and, therefore, the excretion of total iodine follows an inverse compound interest law. Indeed, Hertz and his collaborators found this to be true, even when successive urine specimens were collected and analyzed at short intervals.

Gross Accuracy of the Method. It was difficult for Hamilton and Soley to recover all of this iodine quantitatively because a certain small amount is always lost in the sweat and is difficult to estimate. Moreover, in the extraction of the radio-iodine from biological material there is usually a loss of about 2 per cent of the radio-iodine; and actually the measurement of the radioactivity alone may involve an error of some 3 per cent. Consequently, a recovery of 90 per cent is about all that the technique can offer at present. Techniques are available, however, for increasing the accuracy if it is desirable.

As might be expected, urinary excretion is retarded in severe renal damage; accordingly, this complication must be excluded in studying the influence of the thyroid. In patients with thyroid disturbances the previous administration of iodine had not altered appreciably the excretion of labeled iodine.

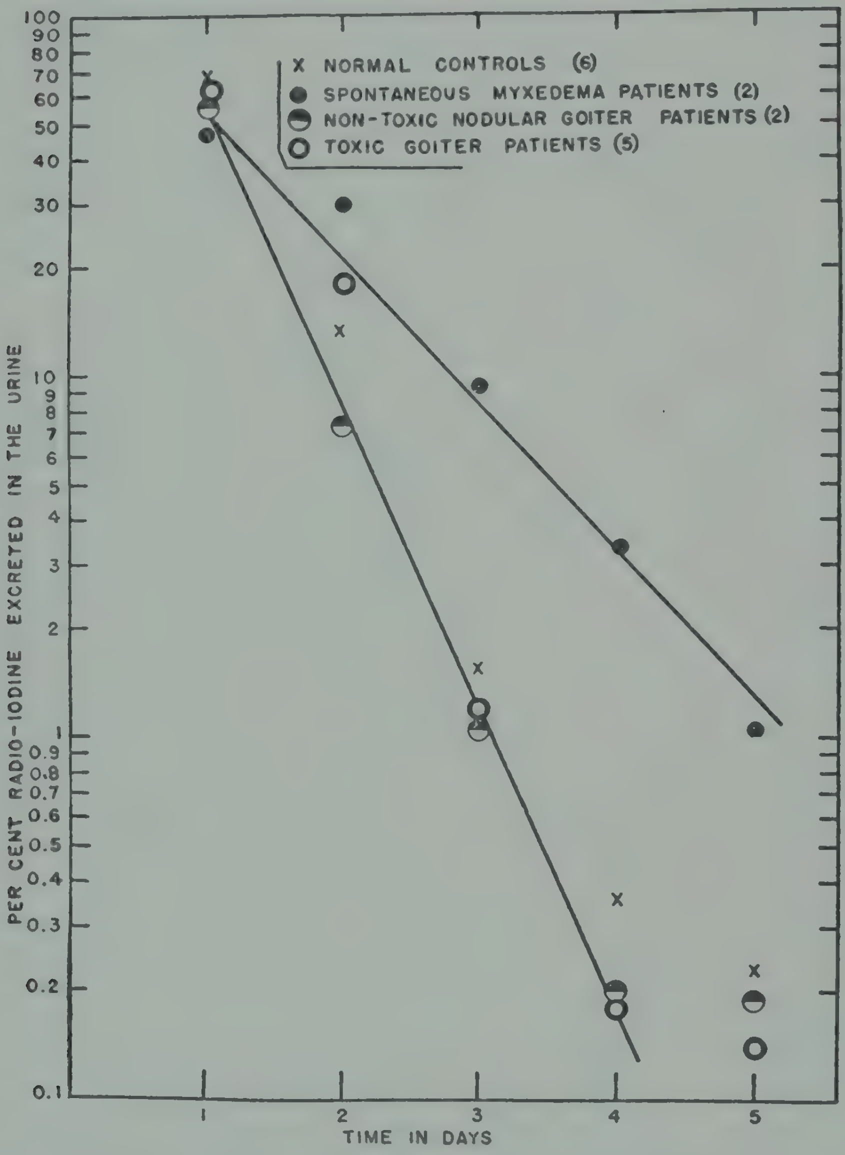


Fig. 31. The urinary iodine excretion for several types of individuals after a single dose of 14 mg. of labeled iodine. Against semi-logarithmic coordinates, the excretion was linear.

From: J. G. Hamilton and M. H. Soley, Am. J. Physiol. *127*: 567. 1939.

RADIOACTIVE IODINE IN THE HUMAN THYROID

In evaluating the amount of iodine found in the thyroid, it must be remembered that although the thyroid concentration is high, nevertheless, the total iodine in the thyroid is only about 20 per cent of the entire iodine in the body; indeed, as mentioned in another chapter, the muscles contain about 50 per cent of all the iodine. This latter amount could be determined by rather heroic biopsies of muscle tissue, which humanity forbids in most instances. In animals, however, it has been shown that non-thyroidal tissue, e.g., skeletal muscle, liver, lymph nodes and thyroid cartilage, fix too little iodine to be measured under circumstances corresponding to these metabolic observations.

Hamilton and Soley [216] have studied in their series of patients not only the absorption and excretion of labeled iodine but also the radio-iodine and total iodine content of the thyroids of patients receiving diluted radio-iodine before thyroidectomy. They have observed, further, the relationship between the microscopic anatomy and the deposition of iodine in histological specimens in the thyroid tissue after surgical removal. They studied 16 patients with toxic goiters, 8 patients with nontoxic goiters, 2 patients suffering from thyroid carcinoma and a patient with "struma lymphomatosa." The gland was removed in each case from 46 to 58 hours after administration of the labeled iodine. The analyses showed that the average uptake of the labeled iodine was about twice as great in the nontoxic goiter as in the toxic variety. Of course, this fact is comprehensible in view of the preliminary routine iodine therapy which the toxic cases received. Nevertheless, these toxic glands, on the average, trapped 8 per cent of the ingested labeled iodine.

When the entire thyroid was removed in each of the patients suffering from carcinoma, it was found possible to separate normal thyroid tissue from malignant tissue and to analyze the two types separately. Likewise, regional metastases were studied. The tumor tissue contained very little of the labeled iodine. On the contrary, the relatively small mass of normal thyroid tissue contained a considerable amount of the radio-iodine. This finding has an important bearing on the possible treatment of thyroid disease with radio-iodine, as described later in this chapter.

Similarly, in a single struma lymphomatosa analyzed after excision relatively little new iodine was found. Apparently the peculiar large cells of which the acini are composed in this condition are incapable of normal function.

The effect of what might be termed iodine hunger on the part of the thyroid was demonstrated by the high radio-iodine uptake in the thyroid glands of the patients with hyperplastic nontoxic nodular goiter. In such glands there was a previous dearth of iodine, but a concomitant hyperplastic follicular epithelium, which was efficient in trapping newly supplied iodine.

The following average figures summarize these differences. In each case the percentage uptake of iodine *per gland* precedes the percentage uptake of iodine *per gram* of thyroid. The respective values are, for toxic goiter, 8.2, 0.13; for nontoxic goiter 16.2, 0.14. In thyroid carcinoma the normal tissue showed 1.2, 0.24 as compared with cancerous tissue 0.13, 0.001 and regional metastases 0.05 and 0.0004. Struma lymphomatosa showed values of 1.8 and 0.013.

Distribution of Radio-Iodine in the Thyroid. In Chapter II the distribution of iodine between colloid and parenchyma was discussed. Hamilton and Soley have studied this problem further by utilizing the effect of beta-rays on photographic film. Paraffin sections from 3 to 5 micra in thickness were cut from excised human glands, then mounted on glass slides and exposed to Agfa "No-screen" x-ray film. After several days the films were developed and the radio-autograph (self portrait) of the section compared with the same section after histological staining. By this technique, in nontoxic goiter the new iodine was found to have accumulated in cellular portions of the gland, but not in the colloid. Thus the smaller acini surrounding the hyperinvoluted areas stored proportionately more of the new iodine. In general, hyperplastic tissue tended to store fresh iodine preferentially. When hyperplasia had been repressed by previous iodide therapy, little fresh iodine accumulated. As might be anticipated from what has been said earlier in this chapter, thyroid carcinoma gave only a faint radio-autograph.

In more recent studies, Hertz and Roberts [241] have performed similar investigations. In these cases, the excised thyroid tissue was analyzed by Salter,[469] who used the original method of Harington and Randall [227] in order to separate the "T" and "D" fractions of the iodine in the gland. In these various cases, which will be discussed in a later chapter, the results followed very closely the curve originally described by Gutman, Benedict, Baxter, and Palmer,[210] based on a study of glands excised at various stages in iodine therapy. It was especially interesting that as the iodine accumulated, in the first stages there was little thyroxine-like iodine; but later, as the iodine approached normal levels, the ratio of "T" to total iodine became fairly constant at about one-third of the whole. Thereafter thyroglobulin was laid down in that constant proportionality, namely, "T":"D" = 1:2 in round numbers.

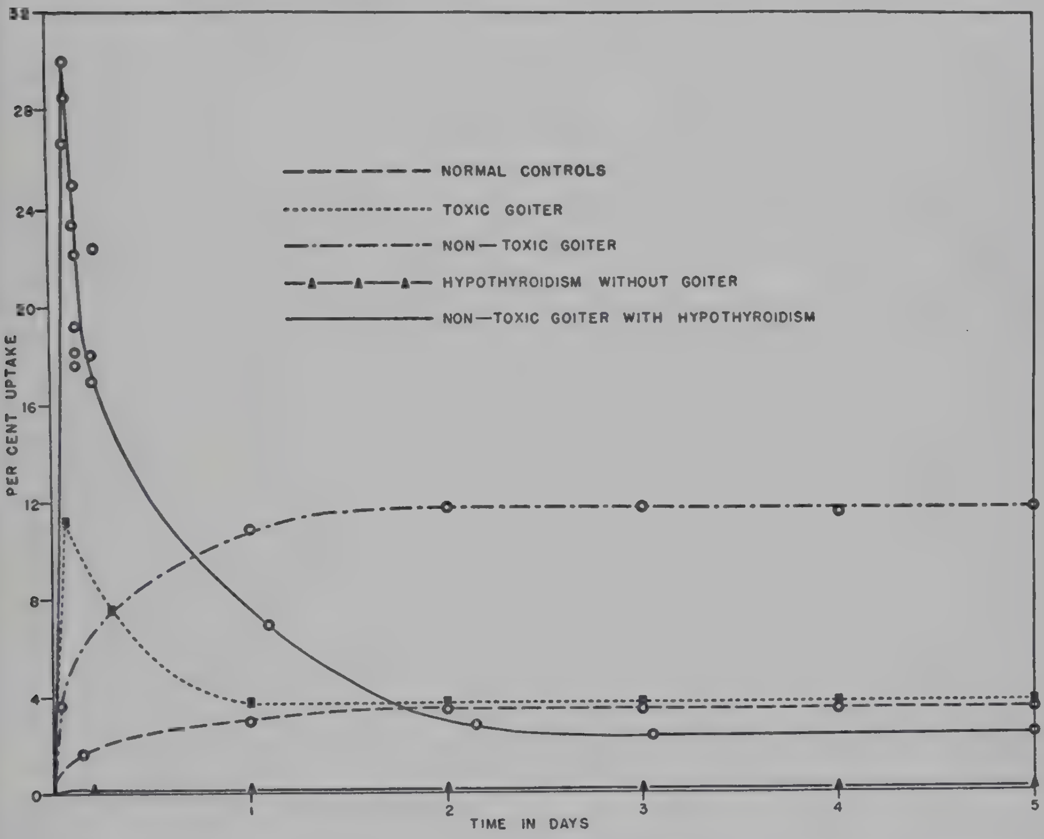


FIG. 32. Direct *in vivo* measurements of labeled iodine in the thyroid of individuals each of whom received a single dose of labeled iodine (14 mg.).

Modified from: J. G. Hamilton and M. H. Soley, Am. J. Physiol. *127*: 568. 1939.

Radio-Iodine Measured in the Human Thyroid in Situ. Of particular interest was the direct study, by means of a Geiger-Müller counter, of the thyroid iodine after treatment with radio-iodine. From day to day the activity emitted by the gland was measured with a Geiger-Müller counter. As shown in Fig. 32, within the first twenty-four hours there was a marked accumulation of activity within the normal gland, which reached a maximum at approximately forty-eight hours. Thereafter no further trapping of radio-iodine could be detected. Subsequently, in the course of five weeks, the loss of radio-iodine from the gland was generally so small as scarcely to be measurable. In this way Hamilton and Soley have studied five normal individuals, five patients with toxic goiter, two patients with nontoxic goiter, one cretinoid child with goiter and concomitant hypothyroidism, and four children with spontaneous myxedema or marked hypothyroidism (without goiter). The nontoxic goiter showed the same general curve as the normal gland, but fixed nearly three times as much iodine. In the toxic gland maximal uptake occurred within one to four hours after administration of the test dose. After twenty-four hours, however, this type of gland contained only one-half to one-fifth of the maximum. Curiously enough, the cretinoid goiter behaved similarly. Thus the glands in these patients showed both an increased avidity for iodine and a disturbance in the mechanism of storage. As might have been anticipated, in the four children with juvenile myxedema, little or no radio-iodine could be detected in the thyroid region. Presumably in such cases the gland is completely atrophic.

In summary, then, these recent studies of newly trapped iodine in human thyroid disturbance reinforce and amplify several principles of thyroid function long recognized by Marine [353] and others.

Critique of Radio-Iodine as a Physiological Method. When an investigator reports unexpected results gained through the use of radioactive materials, the fundamental question is often raised whether a radioactive element has the same biochemical properties as the normal form of the element. Well-informed physicists are almost unanimously agreed that the answer should be in the affirmative, because the radioactive atom does not emit any radiation until the end of its existence, as it changes into another element. Meantime the "tagged" atoms are only potential tracer particles, and show no unusual behavior. To be sure, they have a slightly different atomic weight from that of their fellows, but living tissues appear to be unable to distinguish between mixtures of isotopes to any important extent.

Goudsmit [200] has pointed out also that the presence of the radiation might conceivably alter the properties of the tissues under investigation, and thus affect the outcome of tracer experiments. Ordinarily, however, the radioactive element is so greatly diluted and the radiation so weak, that this effect is very unlikely. The contingency may be examined mathematically, because, were this the case, the yield of the experiment should be roughly proportional to the *square* of the amount of tracer administered instead of directly proportional to it. In general, this fear is worth considering, but is probably unimportant in most instances.

Radiation Therapy with Radioactive Iodine. It has been suggested by a number of investigators that in thyroid hyperplasia and neoplasia it might be possible to treat the disease with iodine, which, being radioactive, would be trapped in the thyroid tissue and would disintegrate there close to the site of the proliferating cells. Hertz, Roberts, Means, and Evans [241] point out that in view of the observations just mentioned, it is possible to calculate the dosage of such material needed for effective therapy. This calculation is based on the assumption that a dose of 100 R units within the thyroid would be desirable. Inasmuch as normal tissues can safely tolerate a dose of one-tenth of an R unit per day continued over the course of many days, the eight-day iodine perhaps would not be appropriate. The twenty-five-minute isotope, however, might well be used to give a short, intense bombardment, quite similar to that obtained in x-ray therapy. In the average rabbit the required dosage to provide 100 R units of radiation in the thyroid is estimated to be about 3 millicuries of the twenty-five-minute isotope. If the human thyroid collected injected iodine only as well as the normal rabbit's, the corresponding dose in human patients with 75 grams of thyroid tissue would amount to about 750 millicuries. In man, however, the hyperplastic gland is several times more efficient, and consequently, less radio-iodine would suffice. Even so, a relatively large amount of radioactive iodine would be needed. Already such quantities are attainable, and such therapy should be tried because it offers such an unusual opportunity for close range bombardment of proliferating tissue which has specifically concentrated the source of radiation.*

Thus, according to this calculation, it might be possible for a brief time, perhaps an hour, to have within the thyroid itself the equivalent of a gram of radium distributed diffusely and emitting radiation of very high intensity!

---

* The millicurie is one-thousandth of a curie, and emits 3.5×10^7 beta-rays per second. This is the amount emitted from one milligram of radium.

*Element No. 85 (Eka-Iodine).** Very recently Corson, MacKenzie, and Segre at the Radiation Laboratory in Berkeley, California have prepared a new chemical element, No. 85. The existence of this substance had long been predicted from such compilations as the periodic table of the elements. Because halogens other than iodine have no great affinity for thyroid substance, it was hardly to be expected that this "eka-iodine" would be trapped preferentially in the gland. Nevertheless, Hamilton and Soley have found that the metabolic behavior of element 85 is similar to that of iodine. Through the courtesy of these investigators the following abstract, prepared personally by Dr. Joseph G. Hamilton, is quoted:

> The recent completion of the 60-inch cyclotron by Ernest O. Lawrence and his associates has made it possible to accelerate alpha particles to sufficient energy to effect a transmutation of bismuth to element 85. This new element is radioactive, has a half-life of 7.5 hours and emits alpha particles. Previous attempts with the smaller cyclotron had been unsuccessful since the alpha particles must possess an energy of above 20 million volts to produce the element 85.
>
> The element 85 was prepared by the alpha-particle bombardment of a water-cooled metallic bismuth target. Following the bombardment, from 100 to 200 milligrams of bismuth were scraped from the surface of the target and placed in a small molybdenum boat in an evacuated chamber. The boat was heated to approximately 400° C. by the passage of an electric current and the volatilized element 85 collected on a plate of glass suspended above the molten bismuth. The element 85 was removed from the glass plate by washing it with carbon tetrachloride, which was then extracted with a dilute alkaline solution of sodium thiosulfate in order to obtain this element in an aqueous solution. Radio-iodine was also used in these experiments and was prepared by the technique employed in our previous studies with the exception that no inert iodine was added as a carrier; therefore the solution of radio-iodine contained less than one-tenth of a microgram of iodide per cubic centimeter. The content of both of these radio-elements was determined for each solution.
>
> The investigation of the uptake into the thyroid glands and the rate of excretion of these two halogens was undertaken with a group of normal and thyrotoxic guinea-pigs. Thyroid hyperplasia was induced in these animals by the daily administration for a period of one week of a specially prepared thyrotropic hormone, which was obtained through the courtesy of Dr. Herbert M. Evans and his associates at the Institute of Experimental Biology at the university in Berkeley. In the first experiment, six thyrotoxic guinea-pigs and four normal animals were used. Each animal received identical doses of radio-iodine and element 85. Three thyrotoxic and two normal animals were sacrificed at the end of four hours and the remainder at the end of 20 hours. The urine and feces were collected for all of the animals during this study; and the thyroid gland,

---

* Many chemists and physicists will feel that "eka-iodine" is a poor name because the substance behaves more like a metal than a halogen. It is amusing to find that its biological behavior is perhaps the best reason for regarding it as a member of the iodine family. Consequently, A. Baird Hastings has suggested the name *thyrine*.

blood, lymph nodes, muscle and liver were removed from each animal after it was sacrificed. The content of element 85 and radio-iodine was determined in all of these samples. Synopsis A, given below, indicates the results obtained in this experiment. The same study was repeated with the exception that this was continued for a period of 65 hours, and the results of this investigation appear in Synopsis B, given below. The uptake of radio-iodine and element 85 into the blood, lymph nodes, muscle and liver was less than one-hundredth the amount present in the thyroid glands.

The synopses indicate that element 85 is accumulated in the thyroid gland and is excreted in a manner very similar to that of iodine. Furthermore, it will be noted that the difference of uptake of iodine in the thyroid with relation to the type of animals used and to the time after administration was followed very closely by element 85. These observations strongly suggest that element 85 is bound firmly in the thyroid gland and probably is attached to benzene rings in a manner similar to iodine. The rapid excretion of element 85 is further proof of its halogen characteristics. It might be pointed out here that the content of iodine and element 85 in the feces was due principally to contamination of the fecal material from the urine since it was difficult to secure complete separation of these two in the metabolism cages which were available. The physiological properties of this new element are very striking since chemically in many respects element 85 behaves more like a metal than a halogen.

Synopsis A. Four hours after the administration of iodine and of element 85, respectively, comparative studies were made with normal animals in contrast to thyrotoxic animals. In normal animals, the data for iodine versus element 85 were as follows: for thyroid uptake 8.5 per cent vs. 3.4 per cent; for urinary excretion 12.4 per cent vs. 8.8 per cent; for fecal excretion 0.8 per cent vs. less than 0.1 per cent. In thyrotoxic animals, the corresponding comparative figures were: thyroid uptake, 26.1 per cent vs. 4.2 per cent; urinary excretion 13.0 per cent vs. 16.0 per cent; fecal excretion 1.0 per cent vs. 0.7 per cent.

Similar observations were made at eighteen hours. In normal animals the data for iodine versus element 85 were as follows: for thyroid uptake 16.9 per cent vs. 5.4 per cent; for urinary excretion 37.2 per cent vs. 36.0 per cent; for fecal excretion 17.0 per cent vs. 13.0 per cent. In thyrotoxic animals, the corresponding values were: thyroid uptake 38.3 per cent vs. 10.7 per cent; urinary excretion 25.7 per cent vs. 34.0 per cent; fecal excretion 5.7 per cent vs. 8.7 per cent.

Synopsis B. Data for thyroid uptake were compiled at four hours, at eighteen hours and at sixty-five hours after administration. These figures comprise comparative values for iodine and element 85 in both normal and thyrotoxic animals.

At four hours, normal animals showed 9.3 per cent for iodine vs. 2.9 per cent for element 85. Thyrotoxic animals showed 28.3 per cent vs. 4.3 per cent.

At eighteen hours, normal animals showed 18.5 per cent for iodine vs. 7.1 per cent for element 85. Thyrotoxic animals showed 39.8 per cent vs. 9.7 per cent.

At sixty-five hours, normal animals showed 18.8 per cent for iodine vs. 4.5 per cent for element 85. Thyrotoxic animals showed 31.8 per cent vs. 8.8 per cent.

Thus the cyclotron has provided another substance with which the biological behavior of the thyroid may be studied at successive stages.

CHAPTER XI

CLINICAL PROBLEMS

BECAUSE progress during the last ten years indicates that in the next decade striking advances will be made in the application of fundamental knowledge to clinical problems, it will be profitable now to take account of stock in order to see more clearly what lines of investigation must be pursued intensively. Throughout Chapter X, unknowns have presented themselves on nearly every page, but it has not been obvious which of these had to do with fundamental knowledge, and which promised speedy application in the clinic. It is the purpose of this chapter to elucidate certain of the roles which iodine may play in the treatment of patients presenting problems which are primarily endocrinological in character.

Before proceeding with a classification of such questions, it is necessary to present a working terminology in order that one may think clearly about them. Because there is at present considerable confusion in terminology, which leads to distorted thinking, it is proposed in what follows to adopt the following conceptual scheme.

I. In the extant clinical literature there are repeated examples of very "toxic" cases of exophthalmic goiter in which the blood iodine and iodine excretion were essentially normal. In pointing out the discrepancy, it is not always clear what is meant by "toxicity." It is well recognized [384] that the complicated syndrome of Graves' disease includes certain symptoms not due specifically to thyroidal hyperactivity. Such are the nervous manifestations which persist when the basal metabolic rate has become *minus*, or the characteristic eye changes which may be found even when frank myxedema has ensued.

The difficulty of correlating chemical and clinical phenomena is due to the fact that whereas the *chemical* data relate directly to the rate of utilization of thyroid hormone, i.e., hyperthyroidism, the *clinical* phenomena are in part due (*a*) to hyperthyroidism and in part due (*b*) to some other mechanism.[54]

Among the clinical signs which are most clearly attributable to hyperthyroidism, and which therefore may be utilized as manifestations of increased thyroid activity, are the following: increased pulse rate, loss of weight (particularly when associated with increased appetite), and hypersensitivity to warmth and increased tolerance to cold.

The degree of exophthalmos, on the other hand, is certainly not invariably related to the degree of hyperthyroidism. Indeed, one may actually observe progressive increase in eye signs accompanying a *minus* basal metabolic rate. The various nervous phenomena, although perhaps in part thyrotoxic in their origin, vary so much from case to case as not to constitute a good criterion for predicting chemical findings. For the sake of convenience in what follows, therefore, we shall distinguish the specifically thyrotoxic manifestations from those which may be due to some other mechanism.[385]

II. In the same spirit, when speaking of iodine metabolism, it will be convenient to think of the general level of iodine balance which is concerned in any given problem. These general levels can be divided arbitrarily as follows. (*1*) The *natural* level, by which is meant the situation when the iodine intake and the iodine output are not far from those normally met with under strictly physiological conditions. Even in untreated thyrotoxicosis one would still be working in this general range. (*2*) The *prophylactic* level, by which is meant the general level of iodine intake and excretion which a wise physician would prescribe for the patient in order that he might avoid thyroid disturbance. In general, this would tend to be slightly higher than the natural level. (*3*) The *therapeutic* level, namely, the source of iodine turnover which one would prescribe in classical Graves' disease. (*4*) The *fibrolytic* level, by which is indicated the huge turnover of iodide utilized in treating such conditions as actinomycosis, or tertiary syphilis. In this chapter we shall say very little about the last group, because it falls outside the scope of this volume. It will be clear, however, that the usefulness of iodine therapy and of iodine analyses will be altered by the level at which iodine metabolism is being pursued in any given instance, and that in studying clinical problems from the standpoint of iodine metabolism this general level must always be ascertained. Therefore, the following sections are separated according to the *daily dose* of iodine.

NATURAL LEVEL OF IODINE METABOLISM
(30 TO 200 MICROGRAMS)

As pointed out in Chapter V, it is not yet possible to predict from the determination of the fasting plasma iodine whether or not the patient is suffering from lack of iodine, or from lack of thyroid hormone. In myxedema, there is a tendency for the total iodine to be low, provided the patient is not receiving an unusually high source of exogenous iodine in the ingesta or from some medication. There are indications already that when methods are better developed to measure the "T" fraction of the blood iodine it will then be clearer whether the patient lacks thyroxine-like material in his plasma or not. This is a problem which is still distinctly in an investigative stage. Many cases of classical myxedema, however, show a "P" iodine (that is to say, a protein-precipitable iodine) which is below the lower limits of normal, and is therefore of great assistance in diagnosis. In endemic goiter, or in simple goiter due to a relative iodine lack, both the total blood iodine and the "P" iodine might be well above the lower limit of normal. Indeed the "T" iodine fraction presumably would not be particularly low except in those regions where severe endemic cretinism abounds. In such places, there is good evidence that the tremendous hypertrophy of the gland fails to accomplish its presumable teleological purpose, namely, manufacture of a normal amount of thyroxine-like material, under the handicap of a low iodine intake.

Because of the fact that unsuspected iodide may have been consumed by the patient just prior to the blood analysis, it is desirable usually to analyze for "P" iodine rather than for total iodine. The application of iodine-containing preparations to the skin is often a source of unsuspected iodine intake. In case of doubt, the plasma "I" fraction (e.g., truly inorganic) should be determined. In such instances, both the "I" and "P" fractions may be high. Plasma should be used, as stated in Chapter IV.

Even if urinary iodine were analyzed in a carefully collected twenty-four-hour specimen, it might not reflect myxedema or the relative iodine lack of simple goiter because, as shown in Chapter IX, urinary iodine may tell only half of the story of the daily turnover of the element. On the contrary, the finding of an unusually high urinary iodine under conditions in which the iodine intake was carefully controlled would be very suggestive of hyperthyroidism. Thus, as described in Chapter IX, urinary iodine excretion may be well over twice the normal average, especially in the early stages of hyperthyroid disturbance. Later on in the disease, when the thyroidal stores are exhausted, iodine excretion may again become normal, even though the hormone in the blood is still high; because the organism is able to conserve and use again a considerable amount of iodine liberated from the degradation products of the hormone.

In the case of true hyperthyroidism, whether part of a classical syndrome of Graves or not, there should be an increase of "P" iodine. The finding of an increased total plasma iodine would not be nearly so conclusive as the finding of an increased protein-bound iodine, because such a high level might merely reflect an excess of circulating inorganic iodide. It is to be hoped that easy methods will be available for determining the thyroxine-like, i.e., the "T" iodine in the blood stream, and this probably will be even more distinctive, although the analytical technique is still rather difficult. Nevertheless, the measurement of the "T" iodine, and perhaps also of the "P" iodine, affords a much more direct estimate of the amount of circulating thyroid hormone than any other index we now know. This will be evident from a short series of values recently obtained at the Thorndike Memorial Laboratory in the Boston City Hospital by Miss Sophia M. Simmons, as shown in Table 22, in Chapter IV.

Likewise, in thyrotoxicosis, especially in the early stages, the finding of increased fecal iodine is of great interest, as indicated in Chapter IX. Ordinarily, fecal iodine is rather low, even when considerable amounts of iodide are administered by mouth or intravenously; on the other hand, in definite hyperthyroidism the stools contain an appreciable percentage, i.e., over 20 per cent of the total iodine excreted. The finding of a high fecal iodine would be distinctly in favor of a thyrotoxic condition, especially if any other disturbance prone to produce diarrhea be excluded.

Typical Problems

It is not the purpose of this volume to discuss thyroid disease from a clinical standpoint directly. There are already in existence recent textbooks on this subject, notably that of Means.[384] Representative clinical cases will be outlined, however, merely to show how iodine metabolism may be applied in the clinic and to illustrate the role of iodine under certain circumstances which are of peculiar clinical interest. Perhaps one of the most interesting features of iodine metabolism is its use in the diagnosis of thyroid disease or in the exclusion of hyperthyroidism. To this end, we shall cite the blood iodine findings in ten individuals, recently seen in the Boston City Hospital, in whom the thyroid status was considered.

Case 1. Normal. This patient was a normal twenty-five-year-old white woman. The basal metabolic rate was plus 2. Past history was irrelevant and the physical examination and laboratory findings were normal. This individual serves as the normal control for the series of cases which follows.

Case 2. Simple Goiter with Possible Hypothyroidism. The patient was a seventeen-year-old single, white woman, the daughter of a government official in Switzerland who, after several years' residence in that country, noted a diffuse swelling of the neck which was diagnosed by a Swiss internist as typical simple goiter, with diffuse bilateral enlargement of the gland. Otherwise there had been no symptoms or complaints. The past history was irrelevant. The menstrual history was normal. The patient had been told that the gland would become normal "in time" on prophylactic iodine therapy. She had taken one drop of Lugol's solution each week for about six months. Physical examination was entirely negative except for a moderate swelling of the gland with diffuse symmetrical enlargement. The basal metabolism was minus 10 and the plasma total iodine 9.1 gamma per cent. The erythrocyte count was 3,900,000, the leukocyte count 6,200 and the hemoglobin 70 per cent. Blood sugar and non-protein nitrogen were both negative. The patient was treated with desiccated thyroid, ¼ grain a day, and reported after three months that she was in perfect health. The pulse had risen from 66 to 80 per minute. The thyroid was perhaps a little smaller. The erythrocyte count was now close to 5,000,000, the hemoglobin 80 per cent.

It will be of interest to follow this case during the next year, to see whether or not the addition of thyroid hormone, with an adequate iodine intake, will maintain normal hemoglobin and physical development. It is noteworthy that the blood iodine was within normal limits. The question arises whether or not determination of the "T" iodine would be somewhat low in this case, as suggested by the rather low basal metabolic rate.

Neither of these two individuals experienced generalized systemic discomfort, and the finding of normal values for plasma iodine is consistent with this fact. By contrast, in the presence of marked discomfort, normal plasma iodine may help considerably in the diagnosis, as indicated by the following cases, treated by Dr. A. H. Coons.

Case 3. Anxiety Neurosis (B. C. H. No. 961449). A white widowed American woman, aged fifty-four, was admitted on November 14, 1939 with a chief complaint of nervousness. The present illness had begun with the menopause, six years previously. Since that time she had experienced increasing nervousness, had lost fifty or sixty pounds in weight, but had noticed no increase in sweating, or any tremulousness. The family history and past history were irrelevant. On physical examination there was found a well developed, fairly well nourished woman with a markedly anxious expression. The skin was normal. The thyroid was not enlarged. There was no exophthalmos. Examination of the heart failed to show any enlargement, murmur, or arrhythmia. There was a moderate tremor of the hands, rather coarse in type, the rapidity of which was roughly three oscillations per second. The blood pressure was 180/90.

The laboratory examinations showed the urine to be negative with a specific gravity of 1.026. The hemoglobin was 84. The erythrocyte count was 4,200,000, the leukocyte count 6,000 per cubic millimeter. The Hinton test was negative. The non-protein nitrogen in the blood was 30 mg. per cent. The fasting cholesterol was 174 mg. per cent. Two stools showed negative guaiac tests. Shortly after admission the basal metabolic rate was determined to be plus 34. At the same time the plasma was analyzed for total iodine, which was found to be 7.1 gamma per cent. Two days later the basal metabolic rate was found to be plus 8, and four days after that the basal metabolic rate was plus 1. Roentgenological examination was negative for tuberculosis, or other disease of the lung.

With rest in bed the blood pressure fell to 130/80 mm. Hg and the patient was discharged on the 25th of November with a diagnosis as follows: Anxiety state; mild, hypertensive vascular disease, without cardiac enlargement.

In this case the apparent increase in "basal" metabolic rate probably was due to excitement or nervousness. The low plasma iodine argued against true elevation in basal metabolism. Cardiac decompensation can produce a similar confusion as seen in the next case.

Case 4. Arteriosclerotic and Hypertensive Heart Disease (B. C. H. No. 964840). A white widowed American printer, aged sixty-nine, was admitted to the hospital on December 14, 1939, complaining of shortness of breath. The present illness had begun one year previously, when he began to notice dyspnea on exertion, and edema of his ankles. For a month he had had paroxysmal attacks of asthma, often nocturnal, and had suffered from a chronic cough. Three days before entry these symptoms became markedly aggravated. Except for a history of chronic alcoholism, the past history was irrelevant. Physical examination revealed a slightly obese, overactive, dyspneic elderly male, sitting up on three pillows. The neck veins were distended. Examination of the chest showed dullness at the right base with râles in this area. The heart was slightly enlarged to the left; the rate was 110 at the apex, with grossly irregular pulses at the wrists both in terms of rhythm, and in force. The abdomen was distended with shifting dullness, indicative of fluid. A tender liver edge could be barely made out, one cm. below the right costal margin. There was pitting edema of the legs as high as the knees. The blood pressure was 200/140 mm. Hg. The extremities showed a coarse tremor. There was no exophthalmos, or lid lag.

The laboratory examination showed the urine to be negative except for a few casts and a very slight trace of albumin on one examination. Erythrocyte and leukocyte counts were normal. The blood Hinton was negative. The non-protein nitrogen was 46 mg. per cent. The blood cholesterol was 188 mg. per cent. X-ray of the heart showed marked cardiac deformity characteristic of hypertensive heart disease, accompanied by mild pulmonary congestion. Electrocardiogram showed inverted T wave in Lead 3 and auricular fibrillation.

Because of the marked nervousness and the almost athetoid movements of the patient, together with redness of the face (a sort of salmon-pink color), hyperthyroidism was suspected. Accordingly, on the 19th of December the basal metabolic rate was done and found to be zero. Simultaneously, plasma "P" iodine was determined (protein-bound iodine) and found to be 7.7 gamma per cent. On the 5th of January the basal metabolic rate was again determined and found to be minus 3. The patient was discharged soon thereafter, with a diagnosis of hypertensive and arteriosclerotic heart disease, generalized arteriosclerosis, chronic alcoholism, cardiac decompensation with congestive failure. In this case, although the symptomatology at the bedside suggested hyperthyroidism, the basal metabolic rate argued against this diagnosis, and the blood iodine was normal.

In this last case both the basal metabolic rate and the plasma iodine concentration were incompatible with the bedside impression of hyperthyroidism. In contrast to this case was a somewhat similar one, as follows.

Case 5. Auricular Fibrillation and Congestive Heart Failure (B. C. H. No. 965392). A forty-four-year-old married Lithuanian housewife was admitted to the hospital on December 14, 1939, complaining of "aching around the heart" for four days. The patient would not admit to being ill prior to four weeks previously, when she began to have slight dyspnea on exertion, with palpitation and irregular heart action. No history of orthopnea or ankle edema could be obtained. She had been separated from her husband recently, and had worried a great deal about finances and other problems. On direct questioning there was no history of excessive sweating; the appetite and the weight were said to have been constant for many years. The past history revealed no rheumatic heart disease; indeed, no history of recent illness could be elicited except for chronic varicose veins. Physical examination revealed a thin, anxious woman with very red elbows (Plummer's sign) and distended neck veins. Ophthalmoscopic examination showed tortuous, narrow, retinal arteries. The thyroid was not palpable. The heart had a heaving impulse at the apex. The left border of dullness was 12 cm. to the left of the mid-line and, in fact, reached the anterior axillary line. The right border of dullness was 4 cm. to the right of the mid-line. The pulse rate at the apex was 160 per minute; at the radial it was 130. The rhythm was grossly irregular. At the base, the pulmonic second sound was slightly louder than the aortic second sound. There was a harsh blowing systolic apical murmur. Diastole was clear. The liver could be palpated 5 cm. below the right costal margin; the edge was tender and smooth. No spleen could be felt. Examination of the chest showed rapid respiration, with coarse crackling râles at both bases. The legs showed marked varicosities and gross pitting edema halfway up the lower leg. The blood pressure was 250/130 mm. Hg.

On laboratory examination the urine showed a large trace of albumin on admission, but this cleared as the heart became compensated. Occasional white cells were seen, but no red cells, and a few coarse granular casts disappeared on rest in bed. Sugar was absent. The erythrocyte count was 4,400,000; the hemoglobin was 75 per cent. The white count was 16,000 on admission, falling to

6,400. The blood Hinton was negative. The non-protein nitrogen was 40 mg. per cent; the cholesterol was 112. An electrocardiogram showed auricular fibrillation with premature ventricular beats. The T-wave in Leads 2 and 3 was inverted, with a low origin. X-ray of the chest showed deformity of the heart characteristic of hypertensive heart disease. The internal diameter of the chest was 26 cm.; the total diameter of the heart was 14 cm. Lung fields were clear.

On the 26th of December the basal metabolic rate was done and found to be plus 39 per cent. At the same time, blood plasma iodine was done and found to be 9.7 gamma per cent, i.e., normal. Three days later the basal metabolic rate was again done and found to be plus 37 per cent. On the 3rd of January, however, it was found to be only plus 18 and it subsequently became normal. Under treatment with digitalis and salyrgan, together with prolonged rest in bed, the pulse dropped from 137 to 70 and the pulmonary congestion cleared, as did the edema of the extremities. The patient was discharged improved with a diagnosis as follows: Hypertensive and arteriosclerotic heart disease with auricular fibrillation and congestive heart failure.

This case of cardiac failure showed the elevation in "basal" metabolic rate not uncommonly found during decompensation. Unlike the preceding case, the plasma iodine concentration failed to agree with the rate of oxygen consumption, and the clinical course showed that the iodine was the better guide.[282]

In the following case, studied by Dr. Chester S. Keefer, recurrent hyperthyroidism was suspected 12 years after an operation for this condition.

Case 6. Toxic Adenomatous Goiter. Mrs. F. L. had a subtotal thyroidectomy in 1925 at the age of forty-seven. In 1939, she was admitted to the Evans Memorial on account of symptoms of recurrent hyperthyroidism, hypertension, and generalized osteoporosis. The basal metabolic rate was plus 80 per cent, the blood pressure was 200 mm. Hg systolic and 100 mm. diastolic. By roentgenray examination it was demonstrated that there was a generalized decalcification of the skeleton which was most striking in the bones of the spine. For several years she has been followed carefully and there has been temporary improvement following the use of Lugol's solution; that is, the basal metabolic rate has been reduced repeatedly, she has gained weight, and more recently there has been evidence that she has begun to retain calcium.

The plasma "P" iodine was 18.6 gamma per cent on admission. After treatment with compound solution of iodine the basal metabolic rate fell to plus 19 per cent. At this time plasma "P" iodine was repeated and seemed not to have dropped, i.e., 19.4 was the value found. Further work is needed on this point, but the present evidence indicates that with the exception of florid Graves' disease, the plasma "P" iodine does not characteristically fall under iodide therapy. Indeed, it tends to rise, and this elevation seems to be even more pronounced in euthyroid and myxedematous individuals than in thyrotoxic patients, as stated in Chapter IV, footnote, p. 99.

In this case both the basal metabolic rate and the plasma iodine agreed with the admission diagnosis. The "P" iodine remained elevated after treatment with iodide, even though the basal metabolic rate fell, with simultaneous clinical improvement. This point will be discussed later. In the next case the diagnosis was far from easy, and the plasma iodine concentration was therefore especially interesting.

Case 7. Toxic Diffuse Goiter (B. C. H. No. 963562). A thirty-nine-year-old white married male janitor was admitted on December 3, 1939, complaining of weakness of six months' duration. The present illness had started insidiously with weakness, loss of weight, increased appetite, excessive perspiration, increased frequency of bowel movements, and intolerance to cold weather. This series of events had come on about six months after the patient had had severe, crushing, substernal pain, lasting several hours, diagnosed at that time as characteristic coronary infarction. A week before entry his weakness had become so extreme that he took to his bed, and for three days before entry he had been having precordial pain in paroxysms lasting for five to ten minutes. The past history revealed that the patient had suffered from joint pains at the age of 12, lasting nearly a year, and that he had had diphtheria in childhood. Physical examination revealed a nervous middle-aged man with a moist, warm skin. The patient had very prominent eyes, with infrequent winking, and a definite lid lag. On questioning, however, he said that his eyes had always appeared so. His thyroid could not be palpated. Repeated examination of the heart revealed paroxysmal auricular fibrillation. The heart was enlarged to the left. The pulmonic second sound was split. The liver edge was 2 cm. below the right costal margin, but no peripheral edema of the extremities could be discovered. The blood pressure was 110/80 mm. Hg.

Laboratory examinations were as follows: The urine was negative. The erythrocyte count was 4,800,000 with a hemoglobin of 99 per cent. The leukocyte count was 6,700 with a normal differential count. The stool showed no guaiac reaction. Blood cholesterol on two occasions was 246 and 232 mg. per cent, respectively. The corrected sedimentation rate was 0.4 mm. per minute. A chest plate showed a very slight deformity of the heart, suggesting rheumatic heart disease. The electrocardiogram showed the absence of the R-wave in Lead 4, characteristic of an old coronary infarct.

On the 7th of December the basal metabolic rate was found to be plus 30 per cent, and on the 11th it was repeated and found to be plus 28. At that time plasma "P" iodine was determined and found to be 16.8 gamma per cent. Unfortunately, fecal iodine excretion was not determined. On the 16th, basal metabolic rate was repeated and found to be plus 24 per cent. Simultaneously the plasma iodine was found to be 16.4 gamma per cent.

In the absence of a palpable thyroid, with the history of lifelong prominence of the eyes and with definite cardiac decompensation present, it was difficult to decide whether the increased basal metabolic rate was due to the heart condition, or due to hyperthyroidism in the absence of detectable goiter. The presence of a very fine tremor in the hands indicated slight evidence of nervousness. The definitely elevated protein-bound iodine, twice found, was even more suggestive of thyrotoxicosis. After considerable cogitation it was decided that (*1*) this patient had Graves' disease; that (*2*) the amount of hyperplasia in his thyroid gland was very minimal; but that (*3*) he suffered from both (*a*) definite thyrotoxicity, and (*b*) moderate nervousness. In consequence, a therapeutic trial with iodine therapy was begun on the 18th of December; the patient received 5 minims of compound solution of iodine three times a day. On the 20th the basal metabolic rate had dropped to plus 19 per cent; on the 22nd to plus 7; on the 24th (a week after the beginning of therapy) the basal metabolic rate was 0; on the 28th it had reached minus 2. At this time blood plasma iodine was again done, and the organic protein-bound iodine was found to be 9.8 gamma per cent, that is, normal. On the 2nd of January a subtotal thyroidectomy was done, and on the 9th blood plasma "P" iodine was determined again as 10.0 gamma per cent. The patient had a rapid convalescence, nervousness had disappeared and the cardiac disturbance had ceased. The metabolic rate at discharge, on the 11th of January, was minus 8 per cent.

The following two cases illustrate the low values of "P" iodine found in hypothyroidism (Cases 8 and 9).

B. C. H. No. 966080. A white, single, well-matured, slightly obese girl of sixteen years entered, complaining of persistent inflammation and pain in the left ankle of three weeks' duration.

At the age of nine years, menses had begun and continued regularly at intervals of three weeks. At that age, mammary development and pubic hair had been nearly mature, and the patient had appeared full grown. Physical examination revealed an intelligent, mature woman. Complete ophthalmological examination was negative. The mammae were large, with erectile nipples. There was definitely masculine distribution of pubic hair, together with considerable hair over the sternum. The lateral abdomen showed a few purplish striae. No evidence of suprarenal masses was found. Pelvic examination revealed a vaginal discharge, but a normal uterus and no ovarian tumor. The skin, generally, was rather rough, dry and cold. The hair was coarse and brittle. The blood pressure was 125/90.

The erythrocyte count was 3,900,000 per cubic millimeter. The hemoglobin was 85 per cent (Sahli). The blood cholesterol was 172 mg. per cent. The sugar tolerance curve was normal. The plasma "P" iodine was 4.8 gamma per cent. The basal metabolic rate was minus 27 per cent. Examination by x-ray revealed a normal sella turcica.

This case responded clinically to thyroid extract, as indicated by changes in the skin, increase in mental activity and elevation in the basal metabolic rate. No definite endocrinological diagnosis was made other than "pluriglandular syndrome." In contrast to this rather vague assortment of abnormal findings is the following case of classical myxedema (Case 9).

M. G. H. No. 227433. A twenty-eight-year-old white married woman entered complaining that for eight years she had been gaining in weight. In the past five years she had gained forty pounds.

Physical examination revealed a dry, rough skin. The tongue was enlarged and thick. Speech was slow. There were "fat pads" over the clavicles.

The erythrocyte count was 4,100,000 per cubic millimeter; the hemoglobin was 80 per cent (Tallqvist). Blood cholesterol was 312 mg. per cent. Sugar tolerance curve was not remarkable. The plasma "P" iodine was 4.6 gamma per cent. The basal metabolic rate was minus 43 per cent. The electrocardiogram showed low voltage.

Little difference in plasma "P" iodine can be demonstrated between classical cases of myxedema and the so-called "forme fruste" of the vague polyglandular syndromes. Perhaps eventually a study of the "T" iodine fraction will elucidate this puzzling lack of distinction. Of course, the time interval required to develop completely full blown myxedema may be several years, and this fact per se may explain some of the differences. In addition, the exceedingly small concentrations of "P" iodine in these hypothyroid cases are so close to the overall blank of the technical methods now available that perhaps variation in the blank itself obscures fine differences in the true "P" iodine, as mentioned in Chapter IV.

Finally, at the other extreme, is the rather terrifying state of thyroid storm. This illustrative case has been reserved until the last because it fortunately constitutes a clinical rarity (Case 10).

B. C. H. No. 971348. A single white girl, aged twenty years, entered in coma characterized by intervals of wild delirium. For some weeks she had become progressively nervous and irritable and her eyes had been called "staring." A physician had been questioned over the telephone and arrangements had been made for a determination of the basal metabolic rate. Soon afterwards the patient became comatose.

Physical examination showed a flushed, cyanotic, thin young woman, wildly thrashing her arms about. The eyeballs were only slightly prominent. The thyroid was diffusely enlarged, with marked thrill and bruit. The isthmus could be palpated. The heart rate was 160. The heart sounds were forceful, resembling embryocardia, but without tic-tac quality. The lung fields were filled with moist râles. The blood pressure was 90/40. The temperature was 103° F.

The plasma "P" iodine was 18 gamma per cent. Electrocardiogram showed sinus tachycardia.

The patient was treated with intramuscular digitalis, morphia, tourniquets to the extremities, alcohol sponging, and absolute quiet. Heavy doses of barbiturates were maintained, supplemented with morphia, p.r.n. Nutrition was assisted with intravenous infusions of glucose solution, given very slowly, supplemented with subcutaneous clyses of saline. For two days aqueous sodium iodide was given intravenously in doses of 1 gram. Thereafter, 1 cc. of liquor iodi compositus was given by mouth thrice daily.

TABLE 43

PLASMA IODINE FINDINGS IN TEN CASES

Case No.	Patient	Age	Sex	Diagnosis	Date	Iodine per 100 cc. plasma (micro-grams)	Remarks	B.M.R. (%)
1	S. S.	25	F.	Normal	11/20/39	10.1	Total iodine	
2	A. P.	17	F.	? Hypothyroidism	11/21/39	9.1	Total iodine	−17
3	B. C. H. No. 961449	54	F.	Anxiety State	11/25/39	7.1	Total iodine	+33.9 + 8.2 + 1.4
4	B. C. H. No. 964840	69	M.	Gen. arteriosclerosis; arteriosclerotic ht. dis.; hypertension; senility; alcoholism	12/19/39 12/21/39 1/ 5/40	7.7	Organic iodine, "P"	0 − 3
5	B. C. H. No. 965392	44	F.	Arteriosclerotic and hypertensive ht. dis. with auricular fibrillation and congestive failure	12/26/39 12/29/39 1/ 2/40 1/ 3/40	9.7	Organic iodine, "P"	+39 +37 +18
6	Evans Mem.	62	F.	Toxic goiter	11/22/39	18.6	Total iodine, before Lugol's	+80
					11/24/39	19.4 63.4	Organic iodine, "P" Inorganic iodine, after Lugol's	+19

7	B. C. H. No. 963562	39	M.	Diffuse toxic goiter Coronary ht. dis. Myocardial infarction (old)	12/11/39			+28
					12/15/39	16.8	Organic iodine, "P,"	
					12/16/39	16.4	Organic iodine, "P," before Lugol's	+24
					12/20/39			+19
					12/22/39			+ 7
					12/24/39			0
					12/28/39	9.8	Organic iodine, after Lugol's	− 2
					1/ 9/40	10.0	Organic iodine, "P," after operation	
					1/11/40			− 8
8	B. C. H. No. 966080	16	F.	Pluriglandular syndrome	1/22/40	4.8	Organic iodine, "P"	−27
9	M. G. H. No. 227433	28	F.	Myxedema	1/27/40	4.6	Organic iodine, "P"	−43
10	B. C. H. No. 971348	20	F.	Thyroid storm	2/ 9/40	18.0	Organic iodine, "P"	

The patient remained restless and comatose for three days. Then the pulse rate slowly fell, the temperature became normal, and on the fifth day glucose and fluids could be given by nasal tube. One week after entry the patient was sufficiently improved to take the routine house diet and feed herself.

This patient illustrates a happy outcome of the much dreaded storm, occurring in an untreated case. It is decidedly a rarity, first, because the patient went into storm spontaneously, and, secondly, because she recovered. The mortality in such cases is probably well over 50 per cent.

It should be remarked that the plasma "P" iodine is the highest in this series. No attempt was made to determine the basal metabolic rate.

The findings in these various cases are summarized in Table 43 for comparative purposes. It should be noted that the values given by the method employed are about 2 to 3 gamma per cent higher than the probable absolute truth.

Comment. As one surveys the above table, it is clear that in Cases No. 2, No. 3, No. 4, and No. 5 the determination of blood iodine concentration was a useful laboratory procedure which, in conjunction with the other clinical data, made the diagnosis more certain. It should be repeated that not too much stress should be laid upon the actual numerical values reported here, because they should be gauged on the basis of the normal for the laboratory and region involved. It must also be remembered that the finding of a normal blood iodine is of great value in ruling out a thyrotoxic condition, in the sense of an increased hormone concentration in the blood. Referring, however, to our initial concept of thyroid disturbance, one can see that it is possible to have (*1*) Graves' disease; (*2*) goiter, with the classical findings in the thyroid characteristic of exophthalmic goiter; accompanying (*3*) the functional status of (*a*) no hyperthyroidism, and (*b*) an extremely nervous state. Many clinicians do not realize that this possibility occurs, and therefore tend to lump "toxicity" of the patient under a general heading of "thyroid disturbance." Nevertheless, as pointed out in Chapter IV, both Curtis in Ohio and Perkin in Boston have found that over one-tenth of their cases show a normal blood iodine in the presence of definite Graves' disease, or allied disturbance. These cases, in general, are those in which the disease has been neglected and, in consequence, the iodine reserve of the patient has been depleted, so that the thyroid no longer has the crude material with which to make hormone in excess. Perkin and Hurxthal [431] have pointed out that such cases are likely to recur after therapy.

Although in Europe the term "Jodbasedow" is used to mean the appearance of hyperthyroidism in a patient who was not previously hyperthyroid, or even suspected of excessive thyroid activity, it seems likely to the author that these cases are most numerous in regions where the iodine supply is relatively low and where, in consequence, a hyperactive gland would be unable to manufacture as much excess hormone as it could in a coastal region, rich in iodine. Among other investigators in Austria, Raab pointed out the possible high incidence of deleterious effects in individuals receiving iodine in medication or as iodide salt. The opinion has been held among the Viennese medical profession that the indiscriminate use of iodine was malpractice because it apparently was so prone to induce hyperthyroidism among the inhabitants. In short, one can suspect that the word "Jodbasedow" really means that a latent hyperthyroidism was exaggerated and became manifest when the crude material needed for making hormone in excess became available.

Further evidence of this difference between European and North American goiter has been collected by Hellwig and presented at the Graduate Fortnight of the New York Academy of Medicine in 1939. He pointed out that in North America 70 per cent of goiters become "toxic," whereas in Bern, Switzerland only 4 per cent become toxic. Indeed, 80 per cent of American adults with diffuse colloid goiter ultimately develop thyrotoxicosis. Corresponding to this clinical difference is the pathological finding that the average weight of the thyroid gland in North America is much less than that in Bern, although the average size of the follicles in North American glands is much larger. These striking differences appear rather extreme in the light of figures given by Means [384] for Massachusetts, namely, 13 per cent toxicity. Read's data [451] seem to disagree.

In this regard, Case 2 is particularly interesting because it illustrates the goitrous enlargement characteristic of many inhabitants of an iodine-poor district, in this case, Switzerland. The enlarged gland had evidently managed to produce a normal amount of hormone, as indicated by the blood iodine; and yet, if anything, the basal metabolic rate was on the low side of standard normal. In treating this type of case, the physician should be wary of supplying large amounts of iodine for fear of an exacerbation in the output of hormone. An ideal form of therapy at first would consist in small doses of thyroid hormone itself, because, as shown in previous chapters, the hormone would tend to suppress pituitary activity and set the thyroid at rest. The actual ideal dosage is a matter of dispute, but obviously it should not exceed the normal physiological requirement, namely, about 2 to 3 grains of desiccated thyroid a day, containing roughly 250 to 390 micrograms of iodine in the form of thyroid hormone. Of course the medication could be given either as desiccated thyroid or as thyroxine. This amount, however, is maximal for adults and many clinicians would prefer to start with not more than 20 per cent of this dosage, gradually increasing it if need be. Such therapy could be continued for many weeks, but because the larger doses tend to suppress thyrotropic hormone activity somewhat, some clinicians prefer to use it intermittently. Eventually such therapy can be supplemented by small doses of iodine in the prophylactic range, to be described presently. Not infrequently, under such therapy, the goiter will become smaller in the course of months or years.

In general, doses above 2 grains of United States Pharmacopeial desiccated thyroid per day must be used with considerable caution. This is particularly true in individuals who have no active thyroid tissue of their own, i.e., are athyreotic. Such patients have no physiological buffer mechanism, and can easily be made hyperthyroid. In cretins this is particularly noticeable, and, in fact, the factor of safety between adequate and toxic dosage is very low.[67] On the other hand, certain patients apparently require dosage of over 3 or 4 grains a day in order to maintain them in suitable metabolic balance. Hoskins [254] has recently called attention to the fact that many cases of obscure endocrine imbalance can be helped definitely by using a superabundance of thyroid hormone, meanwhile observing the patient carefully for untoward symptoms of hyperthyroidism. Such cases, of course, have an unusually high iodine metabolism, but it is entirely exogenous. There need be no fear that additional iodide, if used, will neutralize this thyroid medication because, as Segall [503] showed many years ago, the administration of iodine in any form has no antagonistic effect upon the action of thyroid or thyroxine supplied artificially for therapeutic purposes.

Case 7 is interesting because of the pronounced fall in organic iodine as the basal metabolic rate dropped to normal under the influence of compound solution of iodine, U. S. P. Here was definite evidence, over the course of a few days, that the circulating protein-precipitable "P" iodine, i.e., presumably circulating hormone, had definitely decreased. Together with the therapeutic test of the effect of the Lugol's solution, this constituted very satisfactory evidence that the patient really had been suffering from thyrotoxicosis, as well as nervousness; and, on the basis of this evidence, it was thought desirable to remove his thyroid. The effect of using this iodine therapeutically will be discussed below, but at the moment it may be observed that this therapeutic test of iodine has been emphasized by Means [383] as a very important diagnostic procedure, when carefully performed. It is, of course, essential that the "basal" metabolic rate, as nearly as it can be obtained in nervous patients, should have reached a plateau, when plotted against time, before any iodine be administered. Thereupon a sudden further drop is highly suggestive, particularly when the rate falls within the limits given for the cases studied by Thompson and his collaborators.[540]

Prophylactic Level (200 to 1000 micrograms)

Although it is well known that the treatment of simple goiter or colloid goiter often must be surgical, it has been abundantly demonstrated in the last generation that such goiters can be prevented in large measure by the prophylactic use of iodine. Since the striking demonstration of Marine and Kimball (1917),[358] the chief uncertainty in this method has been the amount of iodine which should be used. As stated in another chapter, normal men can be maintained in iodine equilibrium on intakes as low as 20 micrograms a day, although most normal people ingest at least 80 micrograms a day. Graham [202] (1924) advocated 2000 micrograms a day, Eggenberger [132] (1938), from 70 to 140 micrograms per day, and Marine [352] 75 micrograms per day. Means [384] has compromised on one drop of compound solution of iodine a week, containing 7875 micrograms, or 1125 micrograms per day. Inasmuch as this last dose is well above the ordinary amount of iodine produced by the normal thyroid in twenty-four hours, it seems a reasonable dose to use. Of course, the thyroid retraps and uses again a considerable amount of the iodine released each day from the thyroid, so that this amount would presumably be generous. As evidence of the wide discrepancy in opinion as to the amount needed, one may compare the percentages of iodine used in iodide salt in different parts of the world. Of course, the chief reason for limiting iodine to very small amounts is the fear of so-called "Jodbasedow," but this problem has been discounted by Kimball. He found that only 4 per cent of those individuals who had used iodide salt later developed thyrotoxicosis, whereas 56 per cent of similar individuals who did not use iodide salt or other iodine medication later developed thyrotoxicosis.

Kimball[280] also has recently reviewed the efficacy of goiter prophylaxis through iodine in the past generation and has pointed out in a very convincing way the effectiveness of this therapy. Other factors, however, may possibly intervene from a practical standpoint. For example, Joll[264] (1932) reported that in Derbyshire, England, there had been an increase in the number of children showing goiter since the institution of iodine prophylaxis. Possibly diet is partly responsible, as indicated by the development of goiter in Chesney's[84] rabbits. For many years McCarrison[376] maintained that goiter in India was the result of infection, particularly of water pollution; and, as stated elsewhere, it is true that the presence of infection, as well as the presence of pregnancy, will increase the need for hormone and therefore the need for iodine. In pregnancy many clinicians give iodine to women if they show a low metabolic rate, whether or not they have goiter. Indeed, in some patients showing a low metabolic rate the increase in the iodine supply eventually brings the metabolism back to a normal level, and in such cases the gland often diminishes in size.

THERAPEUTIC LEVEL (2 TO 75 MG.)

By comparison with the figures given in the preceding section, the amounts of iodine ordinarily administered by clinicians in the treatment of hyperthyroidism are really huge. It is not uncommon in this day and age for patients to receive 15 minims of compound solution of iodine three times a day, totaling over 375 mg. of iodine. This dose is, of course, a thousand times the normal daily requirement of iodine, and it is indeed striking that usually it leads to no definitely toxic effects, apart from the rare case of hypersensitivity or iodism. Of course, such large doses are not necessary, as shown by W. O. Thompson and his collaborators.[543] They found that 1 minim of Lugol's solution a day was usually ample; that is, less than 12 mg. of iodine a day produce the maximal response in cases of classical Graves' disease. They did find, to be sure, that some patients failed to respond if they were given less than one-eighth of this dose. The dosage at which patients failed to respond corresponds roughly to the prophylactic level which we have just discussed; in short, in order to get the therapeutic effect one must be distinctly above the prophylactic level. Under such circumstances, as illustrated by Cases 6 and 7, cited above, the total blood iodine runs very high mainly due to inorganic iodide. The same thing may be seen in the blood iodine curve of the dogs treated by Perkin and Brown,[429] as shown in Chapter VII. Concomitantly, a high "P" iodine may be present, as described in Chapter IV, footnote, p. 99.

Wallace and Brodie [563] have studied the iodine distribution under these circumstances and find that the material penetrates the various body fluids in high concentration. Indeed, it behaves very much like chloride. It enters the red cells, but it seems not to enter the muscle cells. It enters brain cells less than chloride, as shown in Chapter IV. Under such circumstances there is a rather high peak of iodine in the blood, often above 100 gamma per cent and sometimes as much as several hundred gamma per cent; but the excess of iodine is rapidly excreted under ordinary circumstances if the kidney is functioning properly. Even twenty-four hours after the ordinary dose, however, the blood iodine may be well above normal, as illustrated by the above cases. Fasting values well over 2.0 mg. per cent of inorganic iodide are not uncommon during iodide therapy.

The effect of such iodine therapy on thyroid function has been discussed in detail in Chapter V. In short, these huge doses of iodine produce, as it were, a chemical thyroidectomy. The gland seems not to put out any hormone for over a week, during which time the tissues and blood run down in a semilogarithmic fashion,[57] much as a motor would slow up with the gasoline line suddenly cut off. When the gland is filled with newly stored thyroid colloid, eventually it overflows, as it were, and hormone again escapes into the blood stream. Means [391] has discussed this disturbance in detail, and believes that the hyperthyroid person always is better under iodine therapy than he would be without it. In short, no matter how bad the patient's clinical state, Means believes that he is enjoying a virtual improvement over his potential condition were he without iodine.

Hertz, Roberts, Means, and Evans [241] have recently continued their work with labeled iodine by observing in Graves' disease the fate of a single large dose of labeled iodide, followed by daily doses of ordinary iodide. Their findings suggest that despite the lowered thyroid activity, only a trace of the initial labeled iodine remains in the gland after ten days. They also suggest the possibility that one single large dose of iodide may suffice to produce a satisfactory preoperative therapeutic effect.

COMPLICATIONS OF IODINE THERAPY

In administering iodide to patients suffering from hyperthyroidism, one can make a mistake in dosage at two extremes. If one gives too much, particularly to so-called hypersensitive persons, one will induce symptoms of iodism, ranging all the way from mild coryza or mild acne to severe dermatitis. On the other hand, if one gives too little iodine, as Marine [355] has shown, one can actually induce exacerbations of the disease, i.e., a sort of "Jodbasedow" effect. This situation is illustrated in Fig. 33, taken from Thompson's work,[540] in which it appears that a daily dose of only 1.5 mg. of iodine allows the patient to become worse, both from the standpoint of bedside observation and from the height of the basal metabolic rate. Marine has pointed out that there is a real danger of inducing exacerbations in hyperthyroidism with doses which are not much above the prophylactic level discussed in the preceding section. As seen in the accompanying figure, when the dose was increased to many times the initial dose, that is, to 180 mg. of iodine per day, such a patient responded with a prompt fall in basal metabolic rate and marked relief in clinical symptomatology. This response quite clearly indicates the difference in action of iodine at the prophylactic and at the therapeutic levels.

Of course, one has other complications of iodine therapy to worry about in terms of time, as indicated in Chapter V. It will be remembered that the so-called "escape from iodine" is admittedly an obvious happening; but on the other hand, as Means [388] has stated, there is probably no true, absolute refractoriness toward iodine.

The patient with untreated Graves' disease has probably lost in large part the normal stores of colloid in the thyroid follicles and, in consequence, he is able for some time to store hormone in his gland rather than secreting it into the blood stream. In Fig. 34 is shown such a response in a clinical case both before and after operation. In consequence of this fact, he has a better opportunity for lowering his metabolism, according to the characteristic decay curve previously discussed in Chapter V. Indeed, some clinicians refuse to operate upon a patient who has recently received iodine. Such a case, they believe, should be treated symptomatically for many weeks, until the iodine stores in the thyroid have again become depleted. Then fresh iodization of the gland should be performed

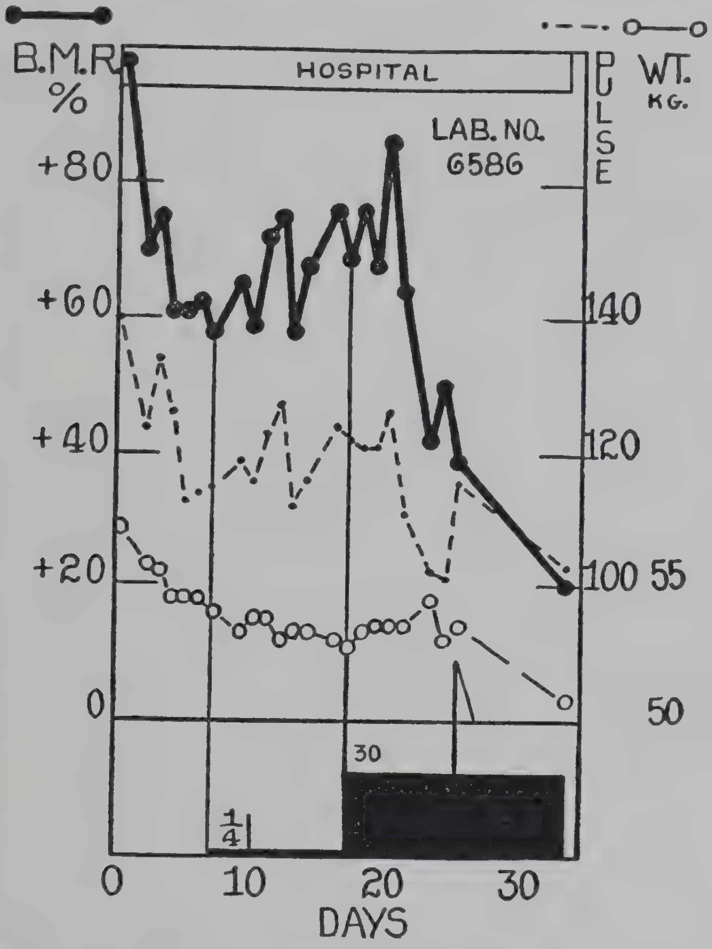


FIG. 33. This graph illustrates the response of a case of exophthalmic goiter to iodine therapy and shows, first, that on a very small daily dose the patient got worse, but that, secondly, on a relatively huge dose the patient subsequently made a characteristic response.

From: W. O. Thompson et al., Arch. Int. Med. *45*: 441. 1930.

and the patient operated upon within a fortnight after iodine therapy has been begun anew. Of course at operation a certain amount of hormone is mechanically liberated, as shown in Fig. 34.

The desirability or expediency of purely medical care in hyperthyroidism will not be discussed in this volume. Suffice it here to point out that under certain selected circumstances, e.g., the hyperthyroidism of acromegaly, or in certain mild types of hyperthyroidism, often the patient may be treated successfully for many years with iodine. Most doctors in modern clinics,[55] however, do not recommend such therapy as a routine; they prefer to have the gland removed, at least subtotally, after a preliminary iodination, as originally preached by Plummer.[141] Ordinarily, the patient's metabolism is allowed to run down as a result of the thyroid blockage

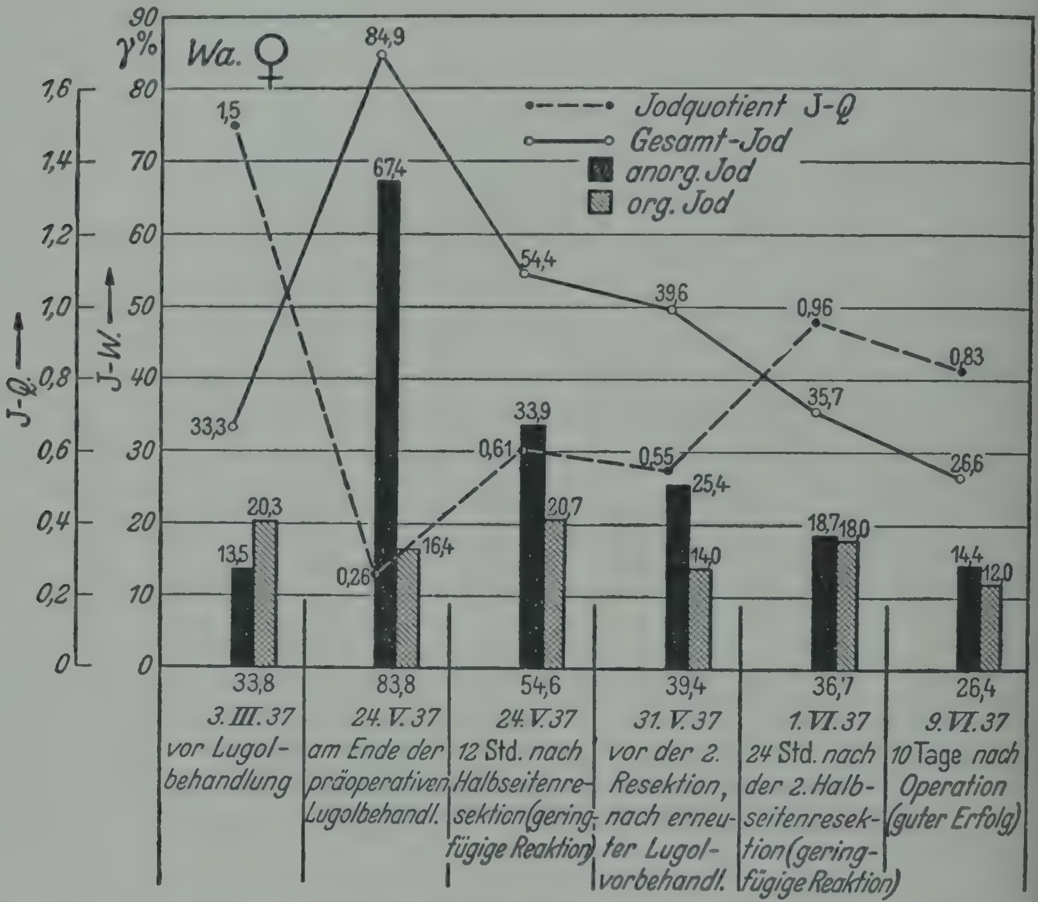


FIG. 34. When a hyperthyroid patient is treated with iodine (e.g., with Lugol's solution), the "organische" blood iodine and the iodine quotient usually fall. Immediately after operation, however, both may rise during the postoperative stormy period. Later both fall generally as the patient recovers.

From: K. Gutzeit and G. W. Parade, Ztschr. f. klin. Med. *135*: 175. 1938–39.

established by massive doses of iodine, and when the maximal effect has been obtained the thyroid is removed surgically, totally or subtotally. If a small remnant of the thyroid is left, the use of iodine after operation is advisable in order to prevent hypertrophy and hyperplasia of this remaining fragment.

Possible Exceptions to Routine Iodine Therapy in Hyperthyroidism. There remain to be settled certain problems which have to do with special cases in the therapy of hyperthyroidism. Outstanding among these are (*1*) cases of Graves' disease with normal plasma iodine; (*2*) cases of Graves' disease with no palpable thyroid and no evidence of anatomical aberrant tissue; and (*3*) cases of mild hyperthyroidism in which operation is refused or contraindicated for some very unusual circumstance. The answer to these problems is not yet known, but there are already certain features of the problems which may be mentioned.[60]

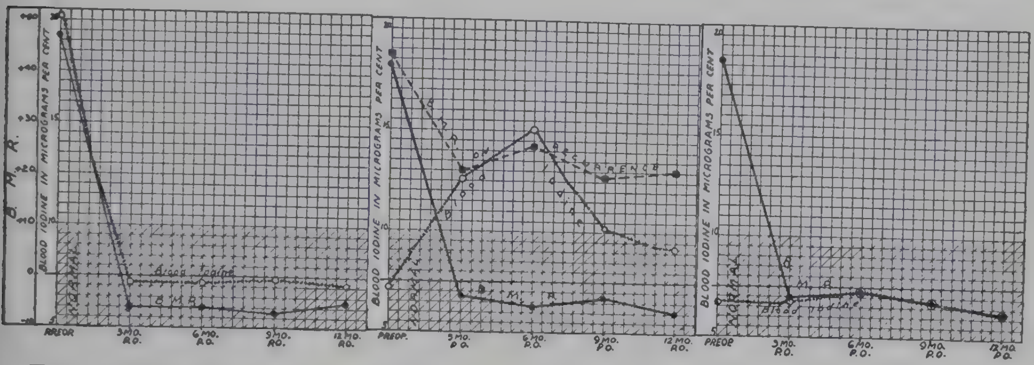


FIG. 35. Three groups of cases have been segregated from 256 cases of exophthalmic goiter. Group 1 (left) represents 170 such patients with an elevated blood iodine level before operation. Most of these made a good recovery after operation. Group 2 (center) illustrates 61 cases with a normal blood iodine before operation but an elevated blood iodine later. Of these patients, nearly a quarter suffered from persistent or recurrent hyperthyroidism or malignant exophthalmos after operation. Group 3 (right) illustrates 25 cases of hyperthyroidism with a normal blood iodine level before operation and a consistently normal level later. These cases did well clinically.

The time in months, preoperative and postoperative, ranging up to 12 months after operation, is shown by the abscissas. The ordinates serve a dual purpose. The basal metabolic rate, ranging from minus 10 to plus 50, is shown by the solid line. The blood iodine in micrograms per cent, ranging from 5 to 20, is shown by the dotted line. The basal metabolic rate on recurrence (center) is shown by a broken line.

From: H. J. Perkin and R. B. Cattell, Surgery, Gynecol. & Obst. *68*: 745. 1939.

1. *Cases of Graves' Disease with Normal Plasma "P" Iodine.* Perkin and Cattell have pointed out that those cases of Graves' disease which have a normal blood hormone in the presence of marked nervousness are very apt to recur. As shown in Fig. 35, their prognosis when seen first is much worse than that of cases with

an increased circulating blood hormone. Treatment in this group is by no means entirely satisfactory, and consequently these cases are of especial interest therapeutically. It would be worth while to study this group from the standpoint of excretion of the thyrotropic hormone. There remains the possibility also that iodination may not be the best form of therapy in this group. Obviously, such problems and such treatment should be considered only in a large clinic, equipped with all modern methods for study and for therapy; but it seems likely that a better form of therapy could be found for this group than is available at present.

2. *Cases of Graves' Disease without Goiter.* It has been pointed out by Naffziger [402] and others that cases of Graves' disease with marked nervousness, but without palpable thyroid tissue or detectable aberrant thyroid tissue, are likely to have continued nervous symptoms even after subtotal thyroidectomy, and, in particular, are likely to suffer from progressive exophthalmos [197] which may be sufficient to endanger vision [403] and demand orbital decompression. The possibility must be entertained that this group should not be treated by thyroidectomy, but that some other form of therapy should be tried (thyroid plus iodide). Studies on the excretion of thyrotropic hormone in this group are badly needed,[356] under conditions in a large clinic where such observations may be suitably pursued.[265] Again in this group, it can be stated categorically that the present form of therapy is by no means satisfactory.[59]

3. *Medical Treatment per Necessitatem.* Every large thyroid clinic accumulates a certain number of cases in which operation, although advisable, is prevented either by the patient's timidity or by some unusual circumstance. In addition, there are a number of cases in which the general systemic symptomatology is so mild that one hesitates to advise surgery for such trifling incapacity. Likewise, after subtotal thyroidectomy symptoms may persist, and such patients must be treated medically, as a rule. Indeed, many internists dream of the day when thyroid disease may be treated by nonsurgical means. Obviously, that day is not yet. Hertz believes, however, that perhaps 5 per cent of all cases coming into a clinic might be treated medically on the basis that their initial metabolic response to iodine therapy goes well below the normal basal metabolic rate. In addition to iodine, roentgen radiation may be applied to the gland itself, as described by Pittman (New England J. Med. 1934).

In a recent discussion on "The Medical Treatment of Hyperthyroidism," Redisch and Perloff [452] maintain that conservative therapy has its place in the treatment of hyperthyroidism, first, because of a certain number of surgical failures, secondly, because some cases are not amenable to surgery and, finally, because they believe that mild cases generally do not need surgery at all. They point out that in Biedl's clinic about 10 per cent of the cases showing favorable results were completely and permanently cured and that another 40 per cent remained entirely free of symptoms as long as iodide therapy was maintained. They add, also, that in Means' clinic at the Massachusetts General Hospital 97.6 per cent of all cases seen from 1923 to 1935 were operated upon, whereas in the last five years the percentage of cases treated with iodine alone has increased to about 14 per cent. They suggest that a larger number of cases could be treated reliably with iodide or with x-ray than is the case at present. They are convinced, however, that iodide therapy should not be given to patients with long-standing nodular goiters which have become "toxic." Thus the pendulum in clinical therapy is swinging back toward a more conservative equilibrium. It is likely that further experience will teach clinicians how to steer between mortality due to thyroid storm and cardiac damage, on the one hand, and surgical accidents and complications, on the other.[60, 408]

It is beyond the scope of this volume to consider such therapy in detail. As pointed out in Chapter IV, following x-ray therapy there may be a mild increase in circulating iodine, presumably as a result of the inflammatory disturbance produced by the radiation. Indeed, occasionally thyroid storm has followed such therapy. Ordinarily, however, after the initial irritation in the gland subsides the organic iodine in the blood plasma decreases and the patient's basal metabolic rate falls.

When iodine is used over long periods as a means of controlling mild hyperthyroidism, there is some evidence that it is better to use the medication intermittently. Thus, at intervals of three months the iodine treatment could be omitted for a number of weeks; during this time the thyroid stores would be depleted, although usually at the expense of some rise in basal metabolic rate. Meantime the clinician hopes that the metabolism will not rise too far because, of course, occasionally even thyroid storm may ensue. As pointed out in Chapter V, if such storm does occur it is customary to give large doses of iodide intravenously. It must be remembered, however, that probably such doses have no neutralizing effect on the hormone in the tissues and their purpose is therefore largely prophylactic in character. If the patient survives the storm for another day or so, such therapy will tend to decrease the amount of hormone reaching the tissues and destined to produce its effect in the near future. Meanwhile, one must have recourse to sedation of various

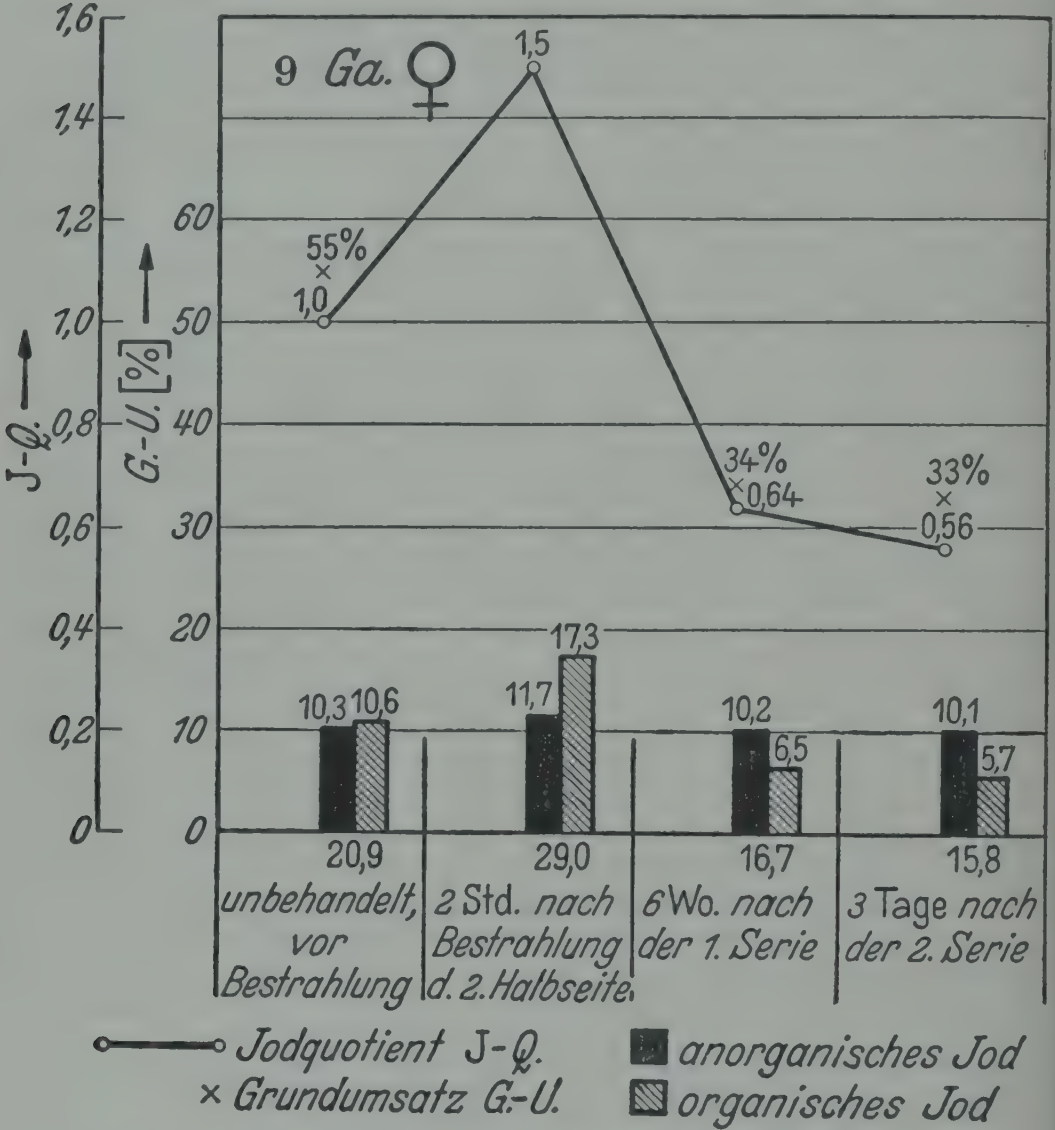


FIG. 36. Even after such gentle treatment as x-ray therapy, there may be an increase in the "organische" blood iodine fraction, together with an increase in the iodine quotient. Both decline as the patient improves and the basal metabolic rate returns to normal.

From: K. Gutzeit and G. W. Parade, Ztschr. f. klin. Med. *135*: 169. 1938–39.

types,[172] maintaining metabolism by high carbohydrate diet as well as possible. Fortunately, this complication occurs only rarely. Very often it is possible for patients to pursue such therapy for many years, and if they do not incur heart damage in consequence, the disease eventually burns itself out without leaving any obvious sequela, with the possible exception of osteoporosis.

The following case will illustrate the response to iodine therapy of a patient seen recently at the House of the Good Samaritan through the courtesy of Dr. Edward Bland.

This case, No. 5684, was that of a twenty-two-year-old married housewife, who six years earlier had been found to have left bundle-branch block in the heart, with a normal P–R interval. As the hemoglobin rose at that time from 45 to 75 per cent, the heart block disappeared, although she still had left axis deviation. Five years before she had again entered the hospital, suffering from known rheumatic heart disease with mitral stenosis and regurgitation, together with aortic regurgitation and stenosis. Following discharge from the hospital she did very well until four months before entry, when she incurred an upper respiratory infection followed by joint pains, precordial pain, headache, and fever at 104° F. A severe exacerbation of these symptoms occurred a month later and the patient was forced to enter the hospital for a few weeks. At that time her doctors told her that the heart was nearly twice the normal size. Ever since, she had suffered from joint pains, severe nosebleeds, and a chronic low temperature. A few weeks before entry another exacerbation of all her symptoms occurred and she was again forced to go to the hospital.

Physical examination showed a well developed, slightly obese, freckled woman, sitting up in bed. On examination of the chest the heart was found to be greatly enlarged; the cardiac-thoracic ratio was 67 per cent. The heart rate was 130 to 140. The apex impulse was very forceful, a split systole being perceptible. There was a systolic murmur audible at the apex; no definite diastolic murmur was heard at this point. To the right of the sternum in the aortic area a harsh systolic murmur was detected. The aortic second sound was absent. A systolic thrill could be palpated in the suprasternal notch. In the presence of fever, the diagnosis was made of active rheumatic fever, rheumatic heart disease, aortic regurgitation, and aortic stenosis.

Because of the disproportion between the low-grade fever and high pulse rate in the absence of definite cardiac arrhythmia, hyperthyroidism was suspected; therefore the patient was subjected to a therapeutic trial of iodine therapy in the form of from 10 to 20 minims of Lugol's solution a day. About five days after the beginning of therapy the pulse rate began to decline, reaching a minimum of about 80 per minute at the end of ten days, and continuing at that level for nearly two weeks as shown in Fig. 37. At the end of this time Lugol's therapy was stopped in order to check its therapeutic effect. The pulse rate began to climb about ten days after the stopping of therapy, and within a fortnight was back at its normal level. When iodide treatment was resumed in the previous dose of 5 minims a day of a saturated solution of potassium iodide, again the pulse dropped 30 points a day within a week.

The sedimentation rate was 1.0, as against the normal of 0.38, a finding compatible with continued rheumatic activity. Under the influence of the iodide, despite the drop in pulse rate, the sedimentation rate remained unchanged and the patient occasionally had mild fever.

In retrospect, careful survey of this history indicates that the patient has had increased nervousness accompanied by tachycardia for nearly a year and a half. The thyroid is about twice normal size, readily palpable, but shows no thrill or bruit, and no nodules have been discovered. The eyes apparently have been slightly prominent for at least five years, but they cannot be considered as exhibiting definite exophthalmos. It is not clear that there is any characteristic fine tremor of the extremities. There has been no diarrhea and the appetite has been poor, but after Lugol's therapy the patient's appetite improved and she felt much less nervous. No obvious change in emotional status has occurred.

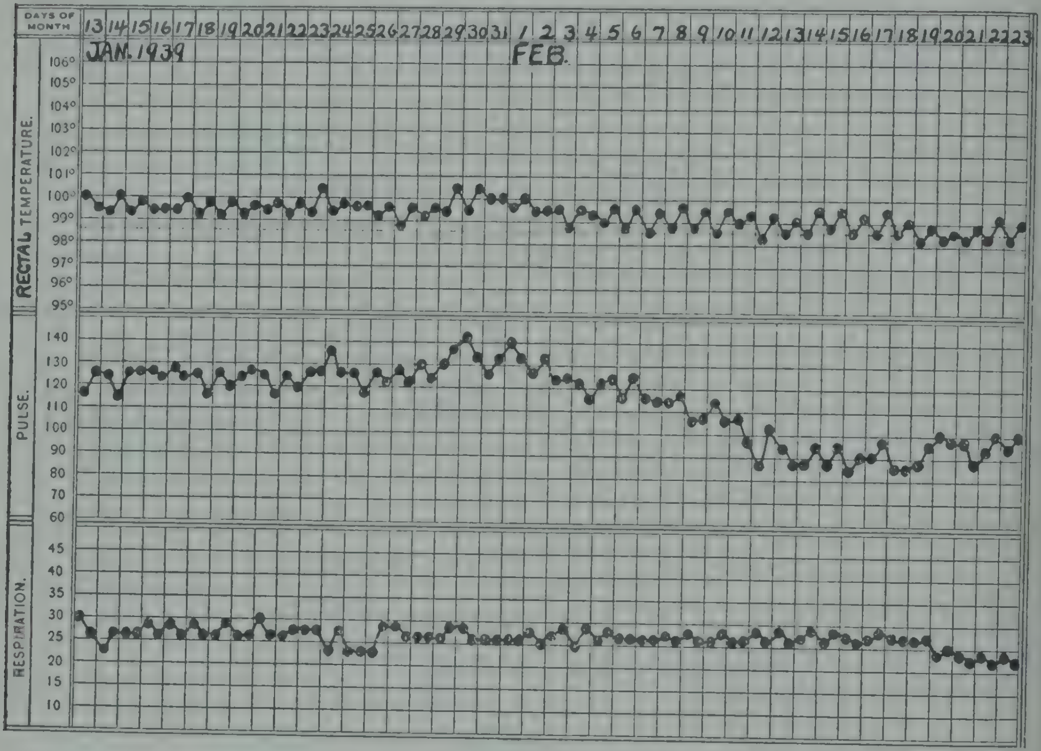


FIG. 37. A characteristic response to a therapeutic test of iodine, shown by the pulse rate, as described in the text. Iodide therapy was started on February 2 and stopped on February 23.

From: House of the Good Samaritan, Boston. By courtesy of the staff.

In view of the present cardiac status with rheumatic activity, and considering the mildness of the symptomatology, it was decided to treat the patient with iodine, possibly combined with x-ray therapy.

She was seen by Dr. J. H. Means, who thought that in view of her active rheumatic infection, and the chronicity of her thyrotoxic state it was wiser to keep her on iodine therapy indefinitely rather than operate upon her at an early date. Indeed, the possibility was entertained that with rest and iodine therapy, supplemented by vitamin B, her thyrotoxic condition could be controlled for the duration of her life.

Poor Planning. One of the grievances of internists who treat hyperthyroidism in a large clinic is that the local physician in charge of the newly admitted case frequently has treated it desultorily with iodine before referring the patient for probable operation.[130] In consequence, the patient is already partially iodinated and often will not make the maximal possible response to preoperative iodine therapy. In some clinics such patients are put on an iodine-free diet; treated only with sedatives for a number of weeks in order that they may exhaust the iodine stores in their glands; and then started afresh with optimal iodine therapy in order to get the best possible metabolic response. This procedure is at present a matter of opinion,* but it is quite clear that small doses of iodine will prevent a maximal iodine blockage of the gland and, in consequence, they probably increase the operative risk somewhat. Furthermore, a few cases treated in this fashion finally arrive at the hospital on the verge of thyroid storm. For these reasons it would seem desirable for practicing physicians before giving iodine therapy to consider whether or not a given case is likely to require operation. If a therapeutic test is to be made with iodine, arrangements should be made first so that the case may be operated upon if the response is positive. Ordinarily, of course, this means hospitalization before any iodine is given. If practicing physicians would look ahead to the possible outcome of thyroid disturbance in their patients, they could spare both the surgeon and the patient a considerable amount of anxiety and discomfort. The vagaries of thyroid disturbance often are unpredictable, of course, but by considering the possibilities in a pessimistic way, one often can give the patient better treatment than would be possible otherwise. In thus laying out a program for the handling of thyroid patients, careful consideration of the possibilities of iodine therapy and its complications is of the utmost importance.[409]

---

* The author believes that if iodine therapy has been started, it should be continued, and supplemented with sedation if necessary.

Iodine Tolerance. Just as in the field of carbohydrate metabolism the glucose tolerance curve may yield valuable information in problems of an endocrinological character, so the iodine tolerance curve may be used in a similar way. Likewise, as with glucose tolerance, there are a number of complicating factors which must be borne in mind always in interpreting such curves.[565] The iodine tolerance curve is very capricious or critical, as pointed out in a previous chapter. As a matter of fact, one can get precisely opposite results from the iodine tolerance by using doses of different magnitude. Nevertheless, if one uses sufficiently small dosage intravenously or by mouth, as shown both by Elmer,[137] and by Perkin and his collaborators,[432] one can find definite evidence that in some cases the thyroid does not trap iodine readily, whereas in other cases the avidity of the thyroid tissue for exogenous iodine is so great that the iodine is "fixed" in the gland almost as rapidly as it arrives into the general circulation. This finding is, of course, completely compatible with the results obtained with radioactive iodine by Hertz and Roberts,[240] and others, as reported in Chapter X.

The iodine tolerance can be used in two ways, just as the sugar tolerance can be tested. The general height of the curve can be estimated from blood iodine analyses at appropriate intervals, as shown in Fig. 38, taken from data of Perkin and his collaborators. It will be seen that in general the normal gland and the nontoxic nodular goiter case do not have enough avidity for iodine to remove the ion from the circulation and so prevent the characteristic peak, which normally rises above an arbitrary limit of about 10 mg. per cent. On the other hand, in Graves' disease or in toxic nodular goiter, the stores of the glands are already so depleted that newly arriving iodide is rapidly seized. In consequence, the peak of the curve is suppressed and the whole curve lies below the arbitrary limit of normal. Unfortunately, this low curve may occur also in hyperplastic glands such as exist in regions of low iodine supply, where iodine starvation prevails. In such instances the low curve *does* indicate that the gland is avid for iodine, but the avidity is due to paucity of iodine rather than to its loss through rapid elimination. The result must be interpreted always with this complication in mind.

It is possible also to use the iodine tolerance test by measuring excretion in the urine. The general principle is the same, namely, that after a standard dose a large percentage of the iodide administered will be excreted in the urine rather rapidly, whereas if the gland be eager to store iodine, much less will appear. Thus Elmer found that after a small dose of iodine nearly 80 per cent would appear in the urine in normal people, whereas only 50 per cent would appear in the urine of hyperthyroid individuals. Because there is a definite time element involved, it seems most convenient to continue these tests over a period of only six hours following the use of a rather small dose, that is, well under 10 mg. of iodine. Otherwise, as pointed out previously, one may obtain exactly the reverse effect. This excretion test can be useful, but it has the added hazard that it is very important to rule out extraneous sources of unsuspected iodine. Furthermore, unusual losses by diarrhea or sweating must be excluded. The advantage of the urinary test, of course, is the avoidance of the laborious technique of repeated blood iodine analyses.

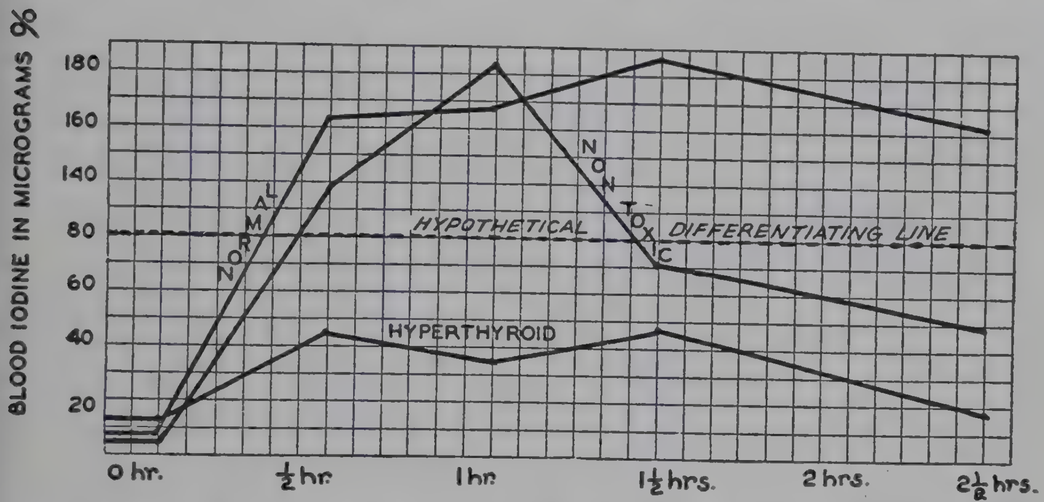


FIG. 38. Blood iodine tolerance curves may be used to separate patients whose thyroids avidly remove iodide from the blood. In New England, most of such patients have suffered from recent hyperthyroidism, in consequence of which the store of iodine in the gland has been depleted.

The curves show the response of blood iodine concentration following the oral ingestion of 6 minims of liquor iodi compositus (U. S. P.) containing 37 mg. of total iodine.

From: H. J. Perkin, F. H. Lahey and R. B. Cattell, New England J. Med. *214*: 51. 1936.

On the whole, considering the complications involved, the iodine tolerance test is probably neither so useful nor so trustworthy as a direct study of the circulating hormone. For this reason, it probably will not be used so much in diagnostic problems. Nevertheless, under special circumstances, particularly in special study wards in large clinics, it probably has a definite place in the unusual case. For example, in those cases of Graves' disease which show a normal blood hormone, preliminary test of iodine excretion followed by an iodine tolerance test with both plasma iodine determinations and urinary iodine excretion for six hours would afford valuable information as to the state of the gland. It must be remembered, however, that other lesions, as pointed out in Chapter VIII, may cause the elimination of excessive amounts of iodine from skeletal muscle or other tissues and so complicate this tolerance test, which therefore cannot be used blindly.

Another type of iodine tolerance test remains to be studied. This is the study of plasma iodine fractions after iodide has been administered continuously for several days. As pointed out in Chapter IV, the plasma "P" iodine will then be found elevated to a varying degree, except in florid Graves' disease. In myxedema, very high values for "P" iodine may be found. It is too early to assess the importance of this chronic tolerance test. (See footnote, p. 99.)

Fibrolytic Level (above 0.5 gram)

After the discovery of iodine by Courtois in 1811, there was a great vogue for iodine therapy. Iodine was used for all sorts of remedies, and indeed was suspended about children's necks in little bags, much like the camphor bag of olden times. In children who were goitrous, this form of therapy probably did some good. Likewise, in the 1820's it was first introduced in the treatment of syphilis, and that use of the medication has continued since. It still is employed in the treatment of various granulomata such as actinomycosis, blastinomycosis, and odd skin disturbances like lupus erythematosus. Occasionally, even today, a gumma is found and the response of such a tertiary luetic lesion to iodide therapy is very surprising, although usually it is customary in these days to use arsenicals or bismuth in addition.

The dosage used in these disturbances is often very high. Doses of several grams a day have not infrequently been administered for considerable periods. Under these circumstances the blood iodine rises to over twenty times the normal, despite rapid excretion by the kidneys. Iodide appears in the salivary secretion and in the mucus of the respiratory tract within a few minutes after being placed in the stomach, and, indeed, all the body fluids are soon permeated with iodide. A great deal of older literature is available on this general subject, but it does not at the moment concern us. Many such references will be found in the "History of Iodine Therapy" by Veil and Sturm.[561]

These huge doses belong distinctly in the realm of pharmacology, or even of toxicology. Indeed, the literature of a generation ago contains repeated cautions against "iodine debauches," calling attention to the incidence of unrecognized chronic coryza, pustules, furuncles, and even emaciation due to the pursuance of iodine therapy in large doses over long periods. This form of therapy, however, still remains important in the treatment of sclerotic lesions of the aorta due to syphilis, and has even been used over long periods for the treatment of generalized arteriosclerosis. One cannot help wondering what complication such therapy may produce in the endocrine system,* but there is available no very clear-cut evidence of manifest endocrinopathy due to these heroic doses of iodine.

---

* The clinical complications of thyroid or thyroxine therapy lie outside the scope of this volume. One instance of endocrine imbalance so produced, however, deserves special note. This is the precipitation of an Addison's crisis in the treatment of myxedema, as mentioned in Chapters III and VI. The phenomenon was described by Castleman and Hertz.[79] In reviewing such cases, Means (Tr. A. Am. Physicians, 1940) concluded that the pituitary was involved, and that the mechanism was related to that of Simmonds' cachexia.

APPENDIX FOR LABORATORY WORKERS

Estimation of Iodine

There is already a large literature on the determination of iodine which has been summarized in detail by McClendon.[377] It is not the purpose of this volume to consider in detail the advantages and disadvantages of the various procedures which are available for adaptation to various types of material. Probably no single method will meet every case without modification. Furthermore, the choice of method often is influenced by such considerations as expense in terms of apparatus required, time involved, analytical training implied, and the type or nature of the material to be analyzed. In addition, every analyst is likely to be swayed by considerations which are not entirely logical. In this appendix, therefore, the author has attempted merely to describe several procedures which will be useful for a considerable number of people over a wide range of iodine concentration. These procedures are presented, not because they represent the maximal accuracy attainable, but because they have proved themselves useful and applicable to a wide variety of biochemical and clinical problems.

Perhaps the most widely adaptable method for making the iodine soluble prior to the analytical procedure is the combustion furnace developed by McClendon and Bratton.[377] This method, however, utilizes a platinum combustion tube into which the sample to be analyzed is fed slowly by clockwork at high temperature. The procedure is quite as formidable as the determination of carbon and hydrogen in organic compounds for the identification of organic structure by professional investigators in the field of organic chemistry. For this reason, it will probably be regarded by many biochemists and clinical investigators as a procedure to which one would have resort as a check on other routine methods.

In addition to the combustion furnace, two other general methods are available for oxidizing organic material and making iodine soluble.[205] The first of these is the method of alkaline ashing in an open crucible. For samples containing over 0.5 mg. of iodine at a concentration greater than 0.1 per cent, this method is very useful and can be relied upon for a wide variety of materials. It has the disadvantage that it is cumbersome as compared with the permanganate oxidation in solution applied to much smaller amounts of iodine, as described below. For even smaller amounts, i.e., down to 0.3 microgram, this "open ashing" has been used by several investigators, including Perkin.[430] It has the following disadvantages: (*1*) the alkaline medium employed may readily fix iodine which is accidentally present in the environment; (*2*) volatilization of sodium or potassium iodide may occur if the temperature is not controlled carefully; and (*3*) the leaching out of the iodide from the inorganic ash is time-consuming and laborious if it is to be done quantitatively. For these reasons the author prefers the so-called "closed" method, which involves the oxidation of the organic material in solution by an oxidant such as potassium permanganate. As a matter of fact, for many purposes it is unnecessary to conduct this oxidation in a closed system; and, as will be described, digestion in an open pear-shaped, or Erlenmeyer flask is adequate. The iodine may then be concentrated by simple distillation, as in the standard micro-Kjeldahl method for nitrogen, if the receiver for the distillate has been prepared previously by the addition of potassium carbonate and sodium bisulfite solutions. Occasionally, combustion may be omitted.[63, 379]

When the organic matter has thus been disposed of and the iodine concentrated as iodide in aqueous solution, the problem becomes one of micro-iodimetry. Many investigators, e.g., Leipert.[309] Fashena and Trevorrow,[166] and McClendon and Bratton,[377] prefer to convert the iodide to iodate with bromine water and then to remove the excess elementary bromine by boiling or aeration.[521] An alternative procedure is the use of the permanganate oxidation suggested by Groák[206] and extensively employed by Matthews, Curtis, and Brode.[369] In this case the excess of permanganate and also of manganese dioxide is removed by treating with nitrite, and the excess of nitrite is removed with urea. Both of these methods finally come to the reduction of the total volume to 2 cc. of solution, in which elementary iodine is released by the addition of potassium iodide. This elementary iodine is then titrated with a microburette, e.g., that of Rehberg, in the presence of starch. An alternative method of titration is the electrometric titration based on the rough measurement of oxidation-reduction potential, as suggested by McClendon.

In order to cover a wide range of needs, four methods for determining iodine are described below. These are (*1*) the Hunter-Kendall alkaline-fusion method for amounts over 0.5 mg.; (*2*) the Groák permanganate method (I) for amounts over 100 micrograms; (*3*) the Groák permanganate method (II) for amounts over 10 micrograms; and (*4*) the Riggs-Man wet-ashing method for amounts above 0.1 microgram. These ranges have been set arbitrarily, because the respective applicabilities of the several methods overlap considerably. Furthermore, the two Groák methods (I and II) are likely to fail if the iodine content of the material under examination is less than 1 per cent. In such a case, of course, one would apply the first or the fourth method, using much more or much less material according to choice.

Amounts above 0.5 mg.

HUNTER-KENDALL OPEN ASHING METHOD. This method is useful for a wide range of materials containing over 0.1 per cent of iodine, provided the material is not volatile. It is well adapted to analyses of thyroid tissue, for example.

(*a*) *Oxidation* is carried out in a nickel crucible of 50 cc. capacity containing 4 cc. of water, 5 to 10 grams of sodium hydroxide pellets and the material to be analyzed. The crucible is supported in a pipestem triangle mounted on a circular iron tripod. The soda is dissolved with cautious warming by means of a small Bunsen burner, and then the heating is continued until the excess of water has been driven off and the mixture, after going through a stage characterized by diffuse frothing, has subsided to a compact melt. During this process some materials will give considerable trouble because of their tendency to boil expansively and overflow. This contingency must be coped with by gentle and intermittent heating, aided by the addition of one or two tiny pinches of finely powdered crystalline potassium nitrate added cautiously with a spatula. Further, more intense heating is gradually applied, but during this fusion precautions must be taken not to volatilize sodium iodide by direct heating of the crucible. This may be avoided either by conducting the strong heating in a deep sand bath, or by using a "soft" flame from a considerable distance. Towards the end of the reaction a few milligrams of powdered potassium nitrate are added successively at intervals of three minutes until no further milky effervescence of the glassy melt occurs.

Because an excess of nitrate will interfere with the subsequent iodimetry, it may be well at this stage deliberately to add a small amount of dry egg albumin from a lot in which the iodine content is negligible, so as to exhaust any extra amount of nitrate reagent. While hot, the crucible is seized with a pair of clean forceps and tipped to an angle of 40 degrees. In this position it is allowed to cool. After 20 minutes it should be cooled enough to handle, when by deft buckling of the crucible the melt can usually be released *in toto*, i.e., "shelled out." The solid melt is transferred to a 500 cc. flat-bottomed conical flask with an extra wide mouth, care being taken not to crack the flask by dropping the heavy melt precipitately into it. The melt is dissolved by adding 200 cc. of distilled water; because this operation requires many minutes it is often convenient to leave the flask stoppered or covered over night at this stage.

(*b*) *Conversion of Iodide to Iodate.* The cool solution is next treated with 2 cc. of 10 per cent aqueous solution of sodium bisulfite, and a minimal amount of methyl orange indicator is added. The alkali is then neutralized with syrupy (70 per cent) phosphoric acid. This neutralization, strangely enough, is the most precarious step in the entire procedure. The flask should be whirled as the acid is slowly poured down the side. Meanwhile the liquor should be kept cool (i.e., below 50°C.). If all goes well, neutralization should be pursued until the solution shows a definite but not deep pink color. Unfortunately, not infrequently the indicator itself undergoes a chemical change so that it falsely shows a yellowish color when the solution is strongly acid; furthermore, under these circumstances it tends to bleach. This complication is more likely to occur in hot solutions, when an excess of nitrate has been used during the fusion or when the process of neutralization has been too long protracted, especially toward the end in the acid phase. After neutralization the solution should be cooled further.

A few drops of elementary bromine are now added and the barest pinch of talcum powder. As the flask is whirled, the bromine first bleaches the dye and then dissolves in the liquid to produce the characteristic yellow of bromine water. At this stage, the spurious yellow of changed dye sometimes obscures the sequence. The solution is then brought to the boiling point and allowed to simmer for eight minutes, a period of time which should suffice to drive off the excess of bromine and leave the solution colorless. While hot, the solution is treated with 10 drops of 5 per cent aqueous solution of sodium salicylate, and then the flask is cooled. This can be done conveniently by inverting a clean beaker over the mouth of the flask and setting it under a running faucet of cold water.

(*c*) *Titration of Iodine.* To the cold solution are added 5 cc. of 10 per cent potassium iodide followed by 5 cc. of 70 per cent syrupy phosphoric acid. The yellow color of freshly liberated iodine should be apparent at once. The elementary iodine is titrated with freshly prepared 0.005 normal sodium thiosulfate, conveniently made by quantitatively measuring 5 cc. of standard 0.1 normal sodium thiosulfate solution into a 100 cc. volumetric flask and diluting to the mark with distilled water. Titration should be pursued until the yellow color is all but gone, and then a few drops of 0.5 per cent starch solution should be added as an indicator. The end point is taken at the last drop which completely removes the blue or purple starch-iodide color for at least one minute. One cubic centimeter of this dilute thiosulfate solution is equivalent to 0.106 mg. of iodine in the original sample.

The following reagents are involved in this method:

1. Sodium hydroxide pellets
2. Crystalline potassium nitrate
3. Soluble egg albumin powder
4. Methyl orange, 0.5 per cent solution in aqueous alcohol
5. Ten per cent aqueous solution of sodium bisulfite
6. Syrupy phosphoric acid, 70 per cent
7. Elementary bromine
8. Talcum powder
9. Sodium salicylate, 5 per cent aqueous solution
10. Potassium iodide, 10 per cent aqueous solution
11. Standard 0.1 normal sodium thiosulfate solution
12. Soluble starch, 0.5 per cent solution

Amounts above 100 Micrograms

PERMANGANATE METHOD OF GROÁK (1).[206] This method will be described for materials containing not more than a trace of bromine, which, if present, requires a modification as described below. The method is not so universally applicable as the Hunter-Kendall procedure, and in general is likely to fail with material containing less than 1 per cent of iodine. Unfortunately, this limitation precludes its use directly for the analysis of thyroid tissue. Nevertheless, the general principle of the method can be applied to very small amounts, as will be shown below; and, after preliminary ashing and concentration of the iodine, may be used for extremely small amounts of iodine. At the moment, a procedure will be described which is applicable to amounts of iodine greater than 0.1 mg., i.e., 100 micrograms. Occasionally, preliminary ashing may be omitted.

About 0.6 gram of anhydrous sodium carbonate and 0.4 gram of powdered potassium nitrate are weighed out and added to the unknown material in a porcelain vessel crucible of 3 by 3 cm. size. One-half cubic centimeter of distilled water is added and the resulting liquor is evaporated to dryness on an asbestos gauze over a small flame. It is then heated with a direct flame until it glows at a dull red and the heating is continued until one can no longer see any dark spots in the melt and the evolution of gas bubbles has subsided. The melt is cooled and dissolved in 5 cc. of hot water. It is then transferred to a 100 cc. Erlenmeyer flask containing a dozen glass beads. The crucible is rinsed into the flask with from 15 to 20 cc. of water, and the neck of the flask is carefully washed down with a little more water. Then 12 to 15 drops of a cold saturated solution of potassium permanganate are added and thoroughly dispersed through the liquor by shaking. The flask is brought rapidly to the boil and allowed to boil for ten seconds. It is then removed from the flame and the hot solution is treated with 5 cc. of a 25 per cent solution of sulfuric acid. This last reagent must be added cautiously at first, drop by drop, because the liquor foams or froths badly through the development of fumes of carbon dioxide and nitrogen dioxide. The reagent can be used to wash down the neck of the flask, at the end.

While still hot, the flask is again heated and treated with potassium permanganate solution which has been diluted to fivefold volume. This dilute reagent is added until a definite rose color persists. The addition of the reagent should be so regulated that the persisting weak rose color should be attained, at the latest, three-quarters of a minute after the beginning of boiling; to achieve this the treatment should start before boiling begins and should be finished as boiling gets under way. After ten to fifteen seconds of boiling the seething solution is treated with exactly 0.1 cc. of 0.1 normal sodium nitrite solution which results in immediate bleaching; at the same time the neck of the flask is quickly rinsed down with water. After twenty-five to thirty seconds' boiling, 1 cc. of a 10 per cent aqueous solution of urea is added and again the neck of the flask is well rinsed with a wash bottle. During this entire procedure the flask has been kept over a flame so that uninterrupted boiling has been maintained. After another minute the flask is removed from the flame and rapidly cooled under running water. Then 1 cc. of 10 per cent aqueous solution of potassium iodide is added, followed by 4 drops of a 1 per cent solution of starch. The resulting starch-iodide complex is then titrated with 0.01 normal thiosulfate from a microburette. The thiosulfate is made up freshly each day from a standard 0.1 normal solution. For larger amounts one can use dilutions at 0.02 normal or even the undiluted 0.1 normal stock solution. In this method 1 cc. of the 0.01 normal thiosulfate is equivalent to 0.212 mg. of original iodine.

In the presence of bromine, free bromine vapor is liberated by the permanganate with the formation of manganese dioxide. Accordingly, after acidification with the sulfuric acid, the liquid must be treated with permanganate solution in excess before the beginning of heating. When gentle boiling begins, further permanganate is added until the muddy brown liquid assumes a definite violet shade. At this point all the bromine has been liberated and can be boiled out almost instantly. Thereupon, 10 per cent sodium nitrite can be added to the boiling solution and the wall of the flask carefully washed down to remove any adherent particles of manganese dioxide. This modification requires only two or three minutes extra time, and after the bleaching, dilute permanganate can be added and the procedure carried out as described above.

It will be noted that for the relatively large quantity of material involved the permanganate serves to oxidize only the last traces of iodine, because most of the iodine has already been taken care of by the preliminary ashing in the crucible. The later treatment with permanganate serves to oxidize the greater part of the nitrous acid set free by acidification. The rest of the procedure is designed simply to remove rapidly and completely the excess of permanganate and nitrite. Under these conditions iodic acid is not attacked. Because of the rather high acidity, however, the final titration must be carried out immediately after the addition of the potassium iodide.

With an aliquot containing 0.5 mg. of iodine, the experimental error is usually under 1 per cent and rarely over 2 per cent, i.e., less than ±10 micrograms.

The reagents used in the above procedure are as follows:

1. Anhydrous sodium carbonate
2. Powdered potassium nitrate
3. Saturated aqueous potassium permanganate
4. Sulfuric acid, 25 per cent solution
5. Diluted potassium permanganate solution (fivefold volume)
6. Sodium nitrite, 0.1 normal aqueous solution
7. Urea, 10 per cent aqueous solution
8. Potassium iodide, 10 per cent solution
9. Starch, 1 per cent aqueous solution
10. Standard thiosulfate, 0.1 normal solution

Amounts above 10 Micrograms

MICRO-PERMANGANATE OXIDATION OF GROÁK (II).[207] Although not universally applicable, for many materials containing more than 1 per cent of iodine by weight, the organic material may be oxidized directly in solution within a few minutes by the following procedure: the material is transformed in weakly alkaline solution at 100°C. into iodate by treatment with a slight excess of potassium permanganate. The excess is reduced in acid solution with sodium nitrite, which in turn is destroyed with urea. Then the iodate is released as elementary iodine by adding potassium iodide and titrated in the presence of starch with 0.001 normal thiosulfate solution. The reagents must all be absolutely free of iodine.

The analysis is conducted in a small flask or a wide test tube (30 by 100 mm.) containing less than 5 cc. of water. After the addition of a drop of normal potassium permanganate solution the tube is placed for two minutes in a boiling water bath. Then 3 drops of phosphoric acid are added, followed by one drop of 0.5 normal sodium nitrite solution. On the addition of the acid the color should at most be faintly reddish, and the added nitrite should produce complete bleaching instantaneously. The wall of the tube is rinsed with the contained liquor, the whole heated in the water bath for thirty seconds and the wall again rinsed. Next, 3 drops of a 40 per cent urea solution are added, the wall of the tube rinsed carefully higher than formerly and the whole heated in the water bath for one minute. After another thorough rinsing of the vessel the tube is cooled. This entire procedure requires about five minutes. Finally, a small crystal of potassium iodide is added, followed by a drop of freshly prepared 2 per cent starch solution.

The following series of routine analyses of an iodopeptide derived from serum albumin were performed by Dr. Jytte M. Muus and the author. For the Groák I method, the solution was diluted to tenfold volume; for the Groák II method, the solution was diluted to one hundredfold volume. Comparative values are given for the Kendall-Hunter method (K.H.) and the two Groák methods (G-I) and G-II). For K.H. 88 gamma, G-I gave 87, 87, 87, 88, and 88; for K.H. 103 gamma, G-I gave 102 and 101; for K.H. 117 gamma, G-I gave 116. Similarly, for a series of K.H. values, i.e., (*a*) 0.70, (*b*) 0.88, (*c*) 1.06, (*d*) 1.06, (*e*) 1.23, (*f*) 1.23, and (*g*) 1.41, the corresponding G-II values were (*a*) 0.78, (*b*) 0.98, (*c*) 1.15, (*d*) 1.07, (*e*) 1.18, (*f*) 1.23, and (*g*) 1.41. These analyses are given to indicate the possibilities of routine work in a clinical laboratory; they do not represent maximal accuracy attainable under ideal conditions.

The starch-iodide complex is titrated with freshly prepared 0.001 normal thiosulfate solution. In this procedure the glass droppers used must be calibrated so that the relative amounts which they deliver are suitably proportioned. In general the method for very small amounts, i.e., under 10 micrograms, should be applied in a volume approaching 2 cc. The preliminary treatment can be conducted in a larger volume and then the water evaporated down after the addition of the urea. In dealing with such small amounts there is obviously a considerable personal equation as regards skill in manipulation. Nevertheless, amounts of 1.5 microgram can be oxidized and titrated with an error of ±4 per cent. Watson[567] describes an apparatus for microtitration.

The reagents required for the Groák (II) method are as follows:

1. Potassium permanganate solution, 1 normal
2. Syrupy, 70 per cent phosphoric acid, specific gravity 1.7

3. Sodium nitrite solution, 0.5 normal
4. Urea solution, 40 per cent
5. Crystalline potassium iodide
6. Starch, 2 per cent aqueous solution
7. Standard, stock thiosulfate, 0.1 normal solution

All of these reagents must be of the highest purity.

Amounts above 0.1 Microgram

WET-ASHING. In the presence of a great excess of organic material, and particularly when the iodine content is less than 1 per cent, this direct oxidation in solution is not applicable. Under such circumstances recourse must be had to a preliminary ashing procedure, followed by some method of concentrating the resulting iodide. A possible method is the ashing of the material in an open crucible in the presence of potassium carbonate. This residue may then be leached repeatedly with alcohol and the combined extracts evaporated in the presence of a small amount of potassium carbonate. The author, however, prefers wet-ashing followed by distillation to concentrate the iodine. A convenient method of doing this is that of Matthews, Curtis, and Brode[369] in which the oxidation is carried out with chromium trioxide in the presence of concentrated sulfuric acid. The excess of the oxidant is removed by treatment with phosphorous acid and the iodine transferred by distillation into a receiver containing potassium carbonate and sulfuric acid. In this case the distillate may then be treated after evaporation, as above described.

Several procedures have been used successfully to concentrate the iodine after destroying the organic material, e.g., in blood plasma or urine, by oxidation. Leipert[312] used chromic oxide, as did Matthews, Curtis, and Brode,[369] followed by distillation. Fashena and Trevorrow[166] used dichromate followed by aeration, hot. The following extension of the permanganate method, however, is very convenient and accurate. It is produced here through the courtesy of the authors.[370]

PERMANGANATE ACID ASHING METHOD OF D. S. RIGGS AND E. R. MAN.*

Apparatus

The *all-Pyrex distilling apparatus* with interchangeable ground-glass joints is drawn to scale in Fig. 39. It consists of the following parts.

(A) *Digestion flask* made from a 900 cc. Kjeldahl flask of which the long neck has been replaced by a ground-glass joint.

(B) *Distilling arm* which connects flask with condenser by two inside ground-glass joints. The capillary tube attached to the dropping funnel extends to within 8.0 mm. of the bottom of flask A. The cross arm from the center of the capillary to the center of the condenser tube is 135 mm. long.

(C) *Short coil condenser*, the water jacket of which measures 170 mm. Below the water jacket is a dew cup.

(D) *Receiving flask* made by cutting off the top of a thin glass, 250 cc., wide mouth Erlenmeyer flask. At the base is joined a short horizontal side arm with a capacity of approximately 3 cc. which is calibrated at 2 cc.

(E) *Thermometer* attached by a heavy rubber band to the capillary tube of the distilling arm. This is 10 cm. long and is calibrated in one degree divisions from 120 to 200°C.

---

* From the Departments of Physiology and Psychiatry, Yale University School of Medicine, New Haven, Connecticut. See J. Biol. Chem., *134*: 193. 1940.

Thermometer. This is 35 cm. long and graduated from 0 to 200°C.

Antibumps. These are 17 and 13 cm. long, respectively. A satisfactory type is made from a short length of glass tubing joined end to end with a glass rod.

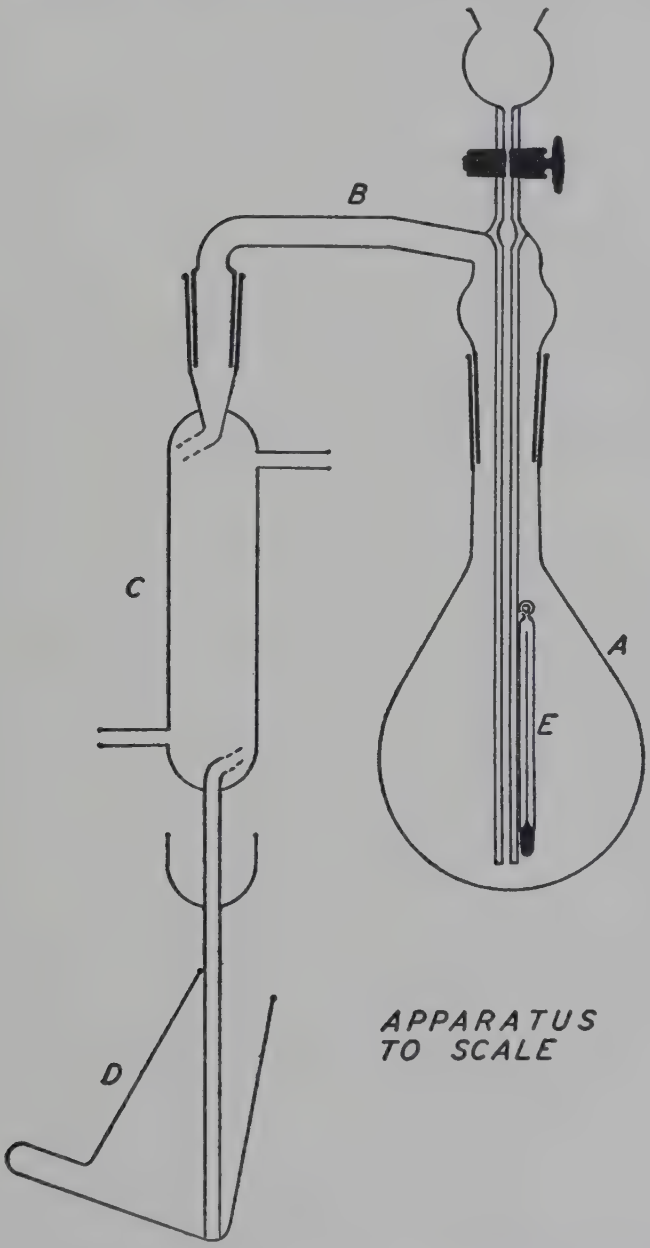


Fig. 39. Diagram of still used in the Riggs-Man method

Fisher Burners.

Electric Hot Plate.

Sand Bath. A Pyrex pie plate covered with a thin layer of iodine-free sand serves as a convenient steady bath for the oxidation step.

Rehberg microburette similar in length and capacity to that described by Rehberg (cf. P. B. Rehberg, A Method of Microtitration, Biochem. J. *19*: 270. 1925.) The tip is attached to the burette by a ground-glass joint and the terminal portion is drawn into a very fine capillary which dips under the surface of the solution during titration.

Wash Bottle. The Corning washing bottle is satisfactory but it should be provided with a saliva trap.

Preparation and Purification of Reagents. Where not otherwise specified, analytical reagent chemicals are used. Redistilled water is used, not only in the preparation of solutions but also to rinse all apparatus. A description of the reagents follows:

(*1*) *Redistilled Water.* Approximately 200 grams of potassium carbonate or hydroxide and a few glass antibumps are placed in a special 12 liter round-bottomed distilling flask mounted on a shallow sand bath. The flask is half filled with distilled water and connected to a water condenser by means of a ground-glass joint. The sand bath is heated and the distillate is discarded until it becomes neutral to methyl red. More distilled water is added to the distilling flask as it is needed. The carbonate should be replenished every two or three weeks.

(*2*) *Potassium Oxalate Solution According to Peters and van Slyke* (*0.1 per cent Iodine Free*). Cf. J. P. Peters and D. D. van Slyke: Quantitative Clinical Chemistry, Vol. 2, Methods. Baltimore. Williams & Wilkins, 1932.

(*3*) *Potassium Permanganate* (Carus, U.S.P.). Seven hundred grams of crystalline potassium permanganate are dissolved as rapidly as possible by heating with 2500 cc. of water. The crystals should be stirred frequently until dissolved. As soon as the last crystals have disappeared, the solution is filtered through glass wool into a beaker and is stirred constantly while the beaker is chilled in ice. The permanganate is suction filtered on Whatman No. 42 filter paper in a Buchner funnel, washed five times with cooled redistilled water, transferred to a large porcelain evaporating dish and dried in an oven overnight at 100°C. It may be necessary to repeat this procedure once or twice.

(*4*) *One per cent potassium permanganate solution* is made from the recrystallized reagent.

(*5*) *Potassium Permanganate Solution* (1 normal). Thirty-one and six-tenths grams of recrystallized reagent are diluted to 1000 cc. with redistilled water. This solution should be stored in a brown bottle in the dark. If a precipitate forms, the solution should be filtered through a glass fritted funnel.

(*6*) *Copper Metal, Granular.*

(*7*) *Ceric Sulfate.* Fifteen grams of ceric sulfate are washed with 75 cc. of boiling 95 per cent redistilled ethyl alcohol. The ceric sulfate is allowed to settle and most of the supernatant alcohol is decanted. The remainder is filtered through a small filter paper. This procedure is repeated twice and the ceric sulfate is then dried in air at room temperature and stored in the refrigerator.

(*8*) *Sulfuric Acid* (18 normal) (Merck or Mallinckrodt, C.P.A.R.; low nitrogen). Concentrated sulfuric acid is poured slowly into redistilled water in the proportion of 17 cc. of acid to 20 cc. of water to make 34 cc. of solution. The sulfuric acid has been found to be essentially iodine free. Boiling the concentrated sulfuric acid for several hours did not alter the blank appreciably.

(*9*) *Sulfuric Acid* (8 normal).

(*10*) *Potassium Carbonate Solution 1 M.*

(*11*) *Sodium Bisulfite Solution 0.1 M.*

(*12*) *Oxalic Acid Solution Saturated at 30°C.* Five hundred grams of oxalic acid (Mallinckrodt) are dissolved by heating to 90°C. in 400 cc. of water. The hot solution is filtered through fluted Whatman No. 1 filter paper into a beaker and is stirred constantly while the beaker is chilled in ice. The crystals are filtered by suction on a Whatman No. 42 filter paper in a Buchner funnel and are washed five times with chilled water. The crystals are kept in the dark at room temperature in a brown bottle with sufficient water to make a saturated solution, saturation being insured by a residue of undissolved acid. Just before use the solution is heated to approximately 30°C.

(*13*) *Sodium Nitrite Solution* (0.75 normal) 1.3 grams per 50 cc. This solution should be made up at least every two weeks.

(*14*) *Urea Solution 5 M.*

(*15*) *Stabilized Arrowroot Starch* (*1 per cent solution*). Ten grams of arrowroot starch are rubbed up with a little water and poured, with stirring, into 1000 cc. of boiling water. The solution is removed from the flame at once and allowed to cool. One gram of salicylic acid is added as a preservative. The solution will keep indefinitely in the refrigerator but should be centrifuged before use.

(*16*) *Potassium Iodide Solution* (*0.2 per cent*). This must be freshly prepared daily.

(*17*) *Sodium Thiosulfate Solution* (0.001 normal). Approximately 26.5 grams of sodium thiosulfate are diluted to 1000 cc. and the solution is allowed to stand for two weeks in order that sulfur may be precipitated. This stock solution (approximately 0.1 normal) keeps well if stored in the dark. The dilute solution, one part of stock solution made up with water to 100 cc., should be prepared not less frequently than once every two weeks and kept in the refrigerator. It is standardized as follows: 2 cc. of 0.01 normal biiodate solution are pipetted into each of two titration flasks, 1 cc. of 1 per cent potassium iodide solution is added and the flasks are cooled in ice water. Ten drops of 8 normal sulfuric acid are added and the solution is immediately titrated with 0.001 normal thiosulfate solution delivered from a 50 cc. burette calibrated at 0.1 cc. intervals. When the yellow color of free iodine has almost disappeared from the solution, six drops of 1 per cent starch solution are added and titration is continued to the point of disappearance of the blue starch-iodine color. The corrected titers of the two samples should check within 0.1 cc. The thiosulfate factor is obtained by dividing the theoretical titer (20 cc.) by the average of the observed titers. If the thiosulfate factor is too great the concentration of the stock solution should be adjusted by appropriate dilution.

(*18*) *Biiodate* (0.1 normal solution) (according to Peters and van Slyke).

Procedure. This is as follows:

Digestion. Fourteen grams of recrystallized potassium permanganate, a small piece of copper and approximately 10 mg. of ceric sulfate are mixed with 15 to 25 cc. of water in a digestion flask. While the mixture is shaken, 10 cc. of oxalated blood are added. Ten cc. of 18 normal sulfuric acid are then introduced and the flask is again shaken vigorously. A violent foaming reaction takes place, during which no more acid should be added lest the contents boil out of the flask. After approximately five minutes, when the reaction subsides, 200 cc. of 18 normal sulfuric acid are added. Two large antibumps and a 200°C. thermometer are placed in the flask, which is supported on an asbestos wire gauze on a ringstand. Heat from a Fisher burner is applied gently at first and the flask is shaken until boiling commences. Just before this point foaming occasionally is excessive, and it may be necessary to stop heating momentarily and to add a little water from a wash bottle. As soon as the mixture is boiling steadily no further shaking is necessary and the flame may be increased. Digestion is continued until a temperature of 195°C. is reached. The entire heating should take about twenty-five to thirty minutes.

When the digest has cooled to below 100°C., 150 cc. of 1 per cent potassium permanganate solution are added. The flask is reheated to 145°C. during a period of about 15 minutes. Constant shaking is necessary during this reheating in order to prevent bumping. When the digest has again cooled to below 100°C. the thermometer is washed with 25 cc. of water and removed.

Distillation. The digestion flask is attached by means of the ground-glass joint to the distilling apparatus. The receiving flask, containing 1 cc. each of 1.0 *M.* potassium carbonate and 0.1 *M.* sodium bisulfite, is tilted so that the tip of the condenser dips below the surface of the fluid. Heat is applied until the temperature of the digest reaches 138° to 140°C. Saturated oxalic acid solution is added through the dropping funnel slowly enough so that the rate of the evolution of carbon dioxide is moderate and the *temperature does not fall below 135°C.* Complete reduction is indicated by decolorization of the digest, although a few minutes may be required for the last black specks to disappear. At least 2 or 3 cc. of oxalic acid in excess should be added to insure complete liberation of the iodine. A greater excess is inadvisable for the reason that will be mentioned under "oxidation." Until the digest is completely reduced, continuous shaking is necessary to prevent bumping. While distillation is continued slowly, water is added from time to time through the dropping funnel to keep the temperature *between 135° and 145°C.* About 170 cc. of distillate should be collected during at least *thirty minutes.*

The receiving flask is provided with a small antibump. On an electric hot plate or an asbestos mat over a Fisher burner the distillate is evaporated to approximately 20 cc. The antibump is washed down and removed, and evaporation is continued more slowly to prevent spattering until the volume is 6 or 7 cc.

Oxidation (As adapted from Groák).[207] The flask is placed in a shallow 70° to 80°C. water bath on a hot plate and eight drops of 1 normal potassium permanganate solution are added. If the eight drops are decolorized, more permanganate is added until a permanent purple color is attained. If too large an excess of oxalic acid was added during distillation more permanganate is necessary. After four minutes, six drops of 8 normal sulfuric acid are added. Carbon dioxide from the carbonate is evolved but the purple color should persist. After another four minutes the flask is removed from the water bath and 0.75 normal sodium nitrite solution is added dropwise with constant shaking until the solution is water-clear and no specks of manganese dioxide remain. One drop of nitrite is added in excess and the sides of the flask are carefully washed by rotating the flask. Two drops of 5 *M.* urea are added at once and the sides of the flask are again carefully washed. The addition of the nitrite and urea should take about one and one-half minutes. The flask is replaced in the water bath until the solution comes to a volume of about 2 cc. Estimation of the volume is facilitated by the use of a special flask provided at the base with a short side-arm calibrated at 2 cc.

Titration. The flask is chilled in ice water and a drop of 1 per cent stabilized starch solution is added. Six-hundredths of a cubic centimeter of freshly prepared 0.2 per cent potassium iodide solution is pipetted into the solution and mixed by rotating the flask. Titration is effected with 0.001 normal sodium thiosulfate solution delivered from a Rehberg microburette. When the blue starch-iodine color has almost disappeared, the flask is again chilled in ice water. As the end-point is approached, not more than 0.0005 to 0.0008 cc. of thiosulfate should be added at once. A comparison flask containing 2 cc. of water and a drop of starch facilitates accurate estimation of the end-point. During addition of thiosulfate, the tip of the microburette should dip below the surface of the solution which is being titrated.

Blank. The technique for determining blanks is essentially the same as that used for blood, except in two respects. The quantity of oxalic acid should be similar to that required in an analysis of blood. The original digestion must therefore be prolonged to attain a degree of reduction of the permanganate equivalent to that which occurs with blood. Iodine must be added after evaporation of the distillate because the value of the blank is so small that accurate titration is impossible.

Fifteen and one-half grams of permanganate, about 20 cc. of water, a piece of copper, approximately 10 mg. of ceric sulfate, and 210 cc. of 18 normal sulfuric acid are mixed in the manner described for blood. The same amount of anticoagulant as that used for one aliquot of blood should be included. The digest is heated to 195°C., allowed to cool below 100°C. and, after the addition of 100 cc. of water, is reheated to 195°C. Eighty-five cc. of water are used to wash the thermometer. Distillation and evaporation of the distillate are carried out as in the blood method. Before oxidation of the 7 cc. of distillate with permanganate, a small, accurately measured quantity of biiodate solution is added to each flask (0.5 cc. of 0.00005 normal biiodate is convenient). On the same day it is advisable to perform control titrations on the same amount of iodate added to a mixture of 1 cc. of 0.1 *M*. sodium bisulfite and 1 cc. of 1.0 *M*. potassium carbonate and carried through the final oxidation and titration processes.

The value of the blank is the difference between the average titers of the control iodate samples and of the distillates to which iodate was added. It does not exceed 0.0050 cc. of 0.001 normal thiosulfate if the reagents have been carefully repurified.

Discussion. Leipert[309] suggested ceric sulfate as a catalyst in his original chromic acid ashing method and copper is widely used to catalyze digestion in the Kjeldahl method. The results of a few analyses of blood with and without the catalysts did not differ significantly, but enough experiments have not been performed to prove whether the catalysts are essential for the determination of iodine in substances other than blood.

It is exceedingly important to use dilute (18 normal) sulfuric acid. Concentrated (36 normal) sulfuric acid must not be used because the concentrated acid reacts with permanganate, even in aqueous solution, to liberate *dangerously explosive* purple fumes of highly oxidized manganese compounds.

The second digestion with 1 per cent permanganate solution may not be essential because in several instances in which this heating was omitted the results were identical with those from samples of blood heated twice. However, some decomposition products of organic material are liberated during the second heating. Also, in the distillation process, reduction with oxalic acid solution proceeds more smoothly if the digest has been reheated with 1 per cent permanganate.

Since the accuracy of the starch-iodine titration is influenced by a great many factors, consistent results depend very largely on maintaining constant conditions of titration. Some of these factors merit brief discussion. The acidity of the solution must be maintained within fairly narrow limits. Too small a concentration of acid gives low titers, while a large excess gives slightly high values. An acidity of approximately 0.6 normal in the solution to be titrated has been found satisfactory. The amount of potassium iodide added before titration must be carefully controlled. In the presence of the large amount of salts in the solution after the Groák oxidation, a large excess of potassium iodide results in a purple, or reddish-violet, starch-iodine color and an indefinite yellowish end-point. If, on the other hand, an insufficient amount of iodide is added, the iodide-iodate reaction does not go to completion and titers are low. For amounts of iodate iodine up to 3 gamma, 0.06 cc. of 0.2 per cent potassium iodide solution is satisfactory. It is important to cool the titration flasks in ice water before, and at intervals during, titration because the sensitivity of the starch indicator varies inversely with the temperature. The source of light should be of constant strength and not bright enough to produce eye fatigue. A small Eastman x-ray illuminator with a sheet of thin white paper across the screen to soften the light has been extremely serviceable. The maximum depth of color is seen if the receiving flask is held at an angle so that the solution forms a shallow pool.

Even under the most favorable conditions, the starch indicator is never sufficiently sensitive to produce a visible color with minute traces of iodine. Therefore, a small amount of iodine will remain untitrated when the starch-iodine color disappears at the observed end-point. Since this amount varies directly with the volume of the solution, it is necessary to add a small volume correction to the observed titer. It is advisable for each investigator to determine the correction independently, by comparing observed and theoretical titers of known iodine solutions. In this laboratory a volume correction of about plus 0.02 gamma (equivalent to about 0.0010 cc. of 0.001 normal thiosulfate) per cubic centimeter of final volume has been employed.

It seems scarcely necessary to stress the importance of avoiding sources of iodine contamination when determining such small quantities of iodine as are present in blood. Tincture of iodine should never be used in preparing the skin for venipuncture, nor should it be used by any of the laboratory workers. Extreme care should be exercised to keep hands and clothing free from iodine-containing solutions which might inadvertently be transferred to apparatus or reagents. All bottles of chemicals which might liberate free halogens should either be kept securely stoppered or removed from the laboratory.

The amounts of the reagents used must be varied in proportion to the quantity of blood to be analyzed. The following formulae express the quantities required:

Grams $KMnO_4$ = 1.2 (cc. blood) + 2

cc. 18 normal H_2SO_4 = 13.3 (total grams $KMnO_4$ used)

(It is especially important to use sufficient 18 normal sulfuric acid since, if less than the indicated quantity is used, a white precipitate will form during distillation and cause bumping.)

cc. 1 per cent $KMnO_4$ = approximately 10 cc. per gram of $KMnO_4$ originally used but this amount must never be less than 100 cc.

cc. H_2O after second heating = 1 cc. per 8 cc. of 18 normal H_2SO_4.

All determinations including blanks are done in duplicate.

Calculations. $\frac{100}{\text{cc. blood used}} \times \frac{\text{T} - \text{Bl}}{0.0473}$ = gamma of iodine per 100 cc. blood.

T = observed titer corrected for burette calibration, thiosulfate factor and volume correction.

Bl = titer of blank.

0.0473 = cc. of 0.001 normal thiosulfate equivalent to 1 gamma of iodine.

The results shown in Table 44 indicate the recovery of iodine from several different types of chemical compound.

TABLE 44

Iodine Recoveries

Iodine Added		Digested With					
		Reagents alone			Blood		
As	Amounts ( )	Number	Av. recovery %	Av. error %	Number	Av. recovery %	Av. error %
KI	0.25–1.00	24	96	5.5	7	99	8.4
Diiodotyrosine	0.50–2.00	18	91	9.1	..	..	..
Thyroxine	0.37–2.48	..	..	..	12	97	4.6
Iodo-Horse-Alb.	2.11–3.04	2	97*	3.0	2	94*	6.0
"T" Peptone	2.70–3.89	2	90*	10.0	2	91*	9.0
"D" Peptone	1.61–1.79	2	93*	7.0	3	96*	5.3

* Calculated from macro-analytical values from W. T. Salter.

Blood iodine values obtained by the permanganate acid ashing method are considerably lower than those obtained by the majority of other methods. The average blood iodine of 18 normal subjects was 3.1, with extremes of 2.5 and 3.7 gamma per cent. A larger series of normals would probably extend the range somewhat. Values from nine patients in the acute stage of hyperthyroidism averaged 10.5, and ranged from 7.6 to 14.2 gamma per cent. One patient with spontaneous myxedema had a level of 1.6 gamma per cent while another patient with long-standing postoperative myxedema had a level of 0.9 gamma per cent. Further studies are in progress.

ELECTROMETRIC TITRATION OF MCCLENDON. Because the titration of iodine in very small amounts with starch as an indicator involves a considerable visual error and therefore a personal equation which in some methods introduces a blank of 25 per cent, the author prefers the electrometric titration method suggested by McClendon.[377] Any laboratory possessing a d'Arsonval galvanometer for accurate pH determinations can construct the necessary additional apparatus at a cost of only a few dollars.

In this method of titration, successive increments of 0.005 cc. of 0.001 normal thiosulfate are added to the final solution of free iodine, and the first derivative of the slope of the titration curve estimated after each incremental titer. When the slope of the titration curve is maximal, the end-point has been reached. In practice, the readings are plotted, and the precise end-point is found at the peak of a series of readings by graphic interpolation.

The incremental change in electromotive force is measured in a simple cell, consisting of a medicine dropper equipped with two platinum wires, inside and outside. Before each addition of thiosulfate, a sample of the solution is drawn up into the medicine dropper. Then the increment of thiosulfate is added to the main solution, which is thoroughly stirred. Thus the two platinum wires record a difference in electronic pressure or activity due to the additional reduction of the solution. This incremental electromotive force is impressed upon a condenser of 1 or 2 microfarads' capacity. After ten seconds, the condenser is discharged through the galvanometer by operating a double-throw switch. Obviously, the galvanometer deflection is a measure of the incremental electromotive force. Only relative values of this deflection are needed, because it is necessary only to know the point of maximum deflection.

With a little ingenuity, this simple apparatus can be assembled in small compass. Under ideal conditions, 0.3 microgram of original iodine may be titrated within an error of 5 per cent. The following routine values were obtained by Dr. Thomas S. Sappington and the author in repeated titrations of a standard solution containing 0.424 microgram of iodine in the aliquot used: 0.44, 0.48, 0.46, 0.44, 0.46, 0.50, 0.43, and 0.39.

Separation of "T" and "D" Iodine

As stated in Chapter III of this volume, the iodine bound in many organic combinations can arbitrarily be separated into two fractions, i.e., "T" iodine, like thyroxine, and "D" iodine, like diiodotyrosine. The resemblance to the prototype compound in each of these categories is based on solubility and not necessarily on biological activity, although under special circumstances these two properties may run hand in hand. For example, in normal thyroid substance, such as is available for therapeutic purposes, the work of Foster, Palmer, and Leland[179] indicates that the "T" iodine may be a better index of potency than the "D" fraction when the drug is used to induce hyperthyroidism in normal animals. In Chapter V are reported similar data, analyzed by Lerman and Salter in the treatment of a human patient in the hyperthyroid range. Similarly, Dr. Jytte Muus and Dr. Albert H. Coons, working in the author's laboratory, have obtained data which suggest that in the progressive iodination of serum albumin the increase in biological activity is reflected by an increase, *pari passu*, in the "T" iodine. In the latter case, however, the activity is many times less than one would anticipate were the "T" fraction truly all thyroxine. With these limitations in mind, however, the analysis of materials for "T" iodine frequently affords interesting information.

Of the various procedures available for this separation, three have been well tested, i.e., (*1*) the procedure of Harington and Randall,[227] (*2*) the method of Leland and Foster,[315] and (*3*) the modification by Blau.[46] Recently Kassell and Brand[269] have suggested a colorimetric method based on the determination of phenolic groups before and after iodination. This last procedure is very convenient and quite accurate for restricted conditions, but its general applicability in a variety of biological products having thyroidal potency has not yet been tried.

The choice between these various methods is somewhat difficult because all involve compensatory errors. The method of Harington and Randall is the simplest, but it tends to give somewhat too high results for "T" iodine because a certain amount of the "D" fraction remains unhydrolyzed and precipitates along with the "T." The procedures of Leland and Foster, and of Blau afford better hydrolysis, but they suffer from the disadvantage that prolonged hydrolysis destroys a certain percentage of the "T" iodine after it is liberated. In this respect, the more gentle hydrolysis of Blau is preferable. On the whole, however, because the procedure of Leland and Foster has been more widely used and therefore better tested, it will be described below. Furthermore, it has been extended into the micro range, which is a definite advantage.

Determination of Thyroxine-like Iodine and Diiodotyrosine-like Iodine according to Leland and Foster[815]

One and one-quarter grams of desiccated thyroid are hydrolyzed for eighteen hours with 100 cc. of twice-normal sodium hydroxide in a 300 cc. short-neck Kjeldahl flask, fitted with a reflux condenser and heated by a micro burner. The use of a potassium thiocyanate bath is recommended to prevent breaking of the flask due to overheating during the prolonged hydrolysis.

The following extraction procedure is used to obtain separately (*1*) the "D" fraction, dissolved in aqueous alkali, and (*2*) the "T" fraction, dissolved in butyl alcohol.

After cooling to room temperature the hydrolysate is transferred quantitatively to a 250 cc. separatory funnel and shaken *gently* for a few minutes with an equal volume (100 cc.) of butyl alcohol, previously purified by distillation from sodium hydroxide. Violent shaking results in the formation of emulsions which require a long time for separation. After standing for one hour the layers are separated. The aqueous layer is transferred to a second 250 cc. separatory funnel and shaken a second time with 100 cc. of butyl alcohol. After standing again, the layers are separated, and the first and second butyl alcohol fractions are filtered successively through glass wool into a 500 cc. separatory funnel. The funnels are washed with 5 cc. portions of butyl alcohol. The combined butyl alcohol fractions are shaken with an equal volume (210 cc.) of normal sodium hydroxide, allowed to stand, and then separated. The sodium hydroxide layer is transferred to a second 500 cc. funnel, shaken with one-half its volume (100 cc.) of butyl alcohol, allowed to stand, and separated. The butyl alcohol fractions are transferred quantitatively to a 500 cc. flask and evaporated under reduced pressure to a small volume. The residue is transferred quantitatively to a nickel dish and the flask washed with several small portions of butyl alcohol. Five cubic centimeters of 50 per cent sodium hydroxide are added, the solution evaporated on a hot plate until the butyl alcohol is entirely gone, and the residue ashed over a free flame. Care should be taken in ashing to use a low flame and never to allow the dish to become red hot. Ashing is considered complete when the melt has changed from caramel to clear, all bubbling has ceased and the bottom of the dish has become coated over with nickel oxide. Particles of carbon left in the melt need not be completely ashed, since they do not interfere with the determination of iodine.

After cooling, the melt is dissolved in hot water and filtered while hot through a Gooch crucible into a 125 cc. Erlenmeyer flask. After thorough washing with hot water (volume 50 to 60 cc.), two drops of a 1 per cent solution of sodium bisulfite are added, then 50 per cent sulfuric acid until acid to methyl orange with three drops in excess and the solution boiled gently for a few minutes. Three cubic centimeters of saturated bromine water are then added, the solution boiled until colorless, and cooled under running water. Six drops of 90 per cent phenol are added to remove the last traces of bromine, an excess of potassium iodide crystals is added and the solution is titrated with 0.01 normal sodium thiosulfate, using starch as an indicator. This strength of thiosulfate has been found to be stable if made up to contain 5 cc. of normal sodium hydroxide per liter.

In a series of fifty-two such analyses, the mean discrepancy between duplicates was 2 per cent; the maximum discrepancy was 5.6 per cent. (Note: This description is slightly modified from J. P. Leland and G. L. Foster: J. Biol. Chem. *95*: 170–171. 1932.)

Analysis for Plasma Iodine: Directions for Separating "I," "P," "T," and "D" Fractions

To exactly 30.0 cc. of 0.02 normal acetic acid in a graduated 50 cc. centrifuge tube add 10 cc. of plasma drop by drop with continuous stirring. Heat in a bath of cold water which is brought to the boiling point in the course of ten minutes. Meantime stir the diluted plasma continuously to prevent the formation of large curds. Centrifuge for ten minutes at 2,000 revolutions per minute. Remove the clear supernatant liquid as cleanly as possible. This solution contains the "I" (inorganic) fraction, which may be estimated with due allowance for aliquot samples. The protein of whole blood is better precipitated with zinc sulfate and alkali. The clear supernatant liquor is saved for analysis of "I" iodine. The method of Riggs and Man is recommended for this purpose, as described earlier.

The "P" fraction is in the protein coagulum, which first must be washed with 0.02 normal acetic acid (20 cc. portions) one to four times, depending upon the amount of the contaminating "I" iodine. Eventually the entire protein coagulum is digested in the acid oxidizing medium preparatory to determination of its iodine content.

If, however, "T" and "D" are to be determined instead of "P," the protein coagulum is hydrolyzed or peptonized and the iodine-containing material extracted four to six times with 10 to 15 cc. of n-butyl alcohol at pH 1 (hydrochloric acid). Alternatively a small continuously extracting apparatus may be used. The combined butyl alcohol extracts now contain "T" plus "D" fractions. By extraction three times with aqueous one molal potassium carbonate solution, the "D" fraction is removed from the butyl alcohol, which thus contains only the "T" fraction. The aqueous "D" fraction is then analyzed for total iodine by one of the methods outlined previously, preferably by that of Riggs and Man. The "T" fraction is first freed of the butyl alcohol by evaporation *in vacuo* in a boiling water bath. The residue is taken up in dilute aqueous carbonate and then analyzed for total iodine.

Obviously the total iodine of the plasma equals fractions "I" plus "P." Likewise fraction "P" equals fractions "T" plus "D."

This procedure is a modification of "The Microdetermination of Thyroxine in the Thyroid Gland of the New-born," described by W. W. Palmer, J. P. Leland, and A. B. Gutman.[425]

ASSAY OF THYROTROPIC HORMONE [450]

Until recently, most of the biological methods for assay of thyrotropic hormone depended upon histological examination of the thyroid glands of animals previously treated with urine, blood, or tissue extracts in known dosage. The intact adult guinea pig has been used frequently and will tolerate the injection of enough crude urine, as Aron [10] showed, to yield a qualitative result. Hertz and Oastler [239] found the hypophysectomized rat more sensitive and reliable, but even with this rather laborious technique the results were only qualitative and showed no definite effect from normal urine. In recent years, however, Starr and Rawson[519] have applied a microhistometric technique which yields a quantitative result which may be expressed in terms of standard units, as shown in Fig. 40. Furthermore, normal urine produced a measurable effect. This technique has been adapted by Dr. Rulon W. Rawson and the author to young chicks, which are cheaper and readily obtained from large poultry hatcheries. Other investigators, e.g., Smelser,[511] and Bergman and Turner,[43] have attempted to use merely the increased weight of chick thyroids as an index of thyrotropic effect, but in the experience of the author's laboratory, after a painstaking microdissection, the results were disappointing. On the other hand, day-old chicks[103] are convenient material for these observations, and the sensitivity of the method can be increased considerably if, instead of gross weight, microhistometric measurements of thyroid cells are substituted. Indeed, by this expedient the method becomes readily applicable to the estimation of the minute concentrations of hormone found in normal human urine. Eventually, when the follicular cells have approximately doubled in height, the cell height index usually has reached a ceiling, above which larger doses produced no obvious hypertrophy. This phenomenon had previously been described for the guinea pig thyroid by Starr, Rawson, et al.[520] In chicks, as described below, this threshold value was attained near a daily dosage of one Junkmann-Schoeller[266] unit, repeated for five days. Up to one-half Junkmann-Schoeller unit, however, there was a steady progression in measurable hypertrophy.

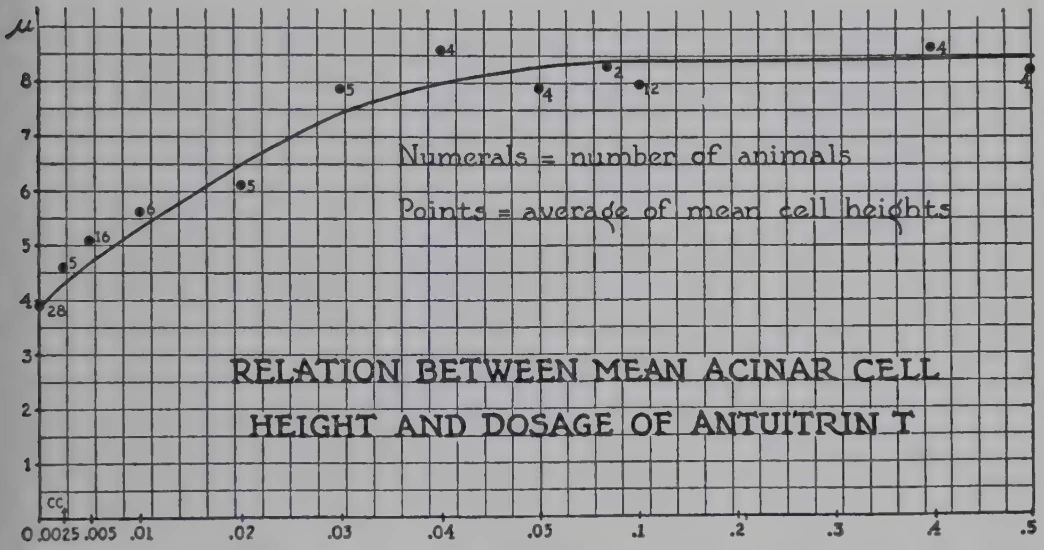


FIG. 40. Nomogram illustrating the relationship between average mean cell heights of groups of animals and the increasing doses of thyrotropic hormone administered to each group.

From: P. Starr et al., West. J. Surg. *47*: 68. 1939.

Of course, the absolute values for cell height vary with the fixation technique, the species and age of chick and other circumstances. It is desirable, therefore, for each investigator to calibrate his total technical procedure by obtaining a nomogram, as shown above. Once this diagram is obtained for a standard preparation of known potency, unknown materials may be assayed similarly and the results expressed in units.

The normal urine of young adults, when tested by this method, contains approximately one-eighth Junkmann-Schoeller unit per 100 cc., or nearly two Junkmann-Schoeller units per day. After thyroidectomy has been performed, several times this amount may be found in human urine. Likewise, in *incipient* hypothyroidism an increase may often be demonstrated, and in partially relieved myxedema the same may be true.

The young-chick method has the disadvantage of not being so specific as assay with the hypophysectomized rat. It is more practicable, nevertheless, and gives a numerical answer. The lack of specificity may be compensated for somewhat by a preliminary partial purification of the material, e.g., urine. Such a procedure has been undertaken by Rawson, and developed further by Coons in the author's laboratory.

The adaptation of Rawson and the author follows:

Method. Day-old chicks weighing from 35 to 40 grams were used as test animals. No attempt was made to separate them according to sex. The animals were kept in a brooder at a constant temperature of 95°F. and maintained on a diet of commercial chick starter mash, containing under 0.01 per cent iodine.

The chicks were labeled and injected daily for five days with thyrotropic hormone in the following doses: one-eighth Junkmann-Schoeller unit, one-quarter Junkmann-Schoeller unit, one-half Junkmann-Schoeller unit, and one Junkmann-Schoeller unit. On the sixth day the animals were autopsied and the thyroids removed and fixed in Zenker's solution. Later work indicated that a shorter period of fixing (at least two hours) may be adequate and produces less shrinkage, because the small diameter of the chick thyroid (e.g., 3 mm.) allows rapid penetration of the fixative. Recently, at the suggestion of Doctors Allan Grafflin and H. L. Weatherford of the Department of Anatomy of the Harvard Medical School, 10 per cent formalin has been used as a fixative with less shrinkage and with more satisfactory sections in consequence.

Paraffin sections were made and stained with hematoxylin and eosin. The histological preparations were then studied under oil immersion. The gland was crossed diametrically and recrossed until one hundred successive clearly delineated acini had been observed. From each of these acini a representative cell was chosen and its height measured with a Leitz echelon micrometer. No cell should be measured unless its limits are clearly visible. From the observed measurements made on each gland a frequency curve was plotted as shown in Fig. 16, and its mean and standard deviation determined.

Results. This method has been tested repeatedly in assays involving several hundred animals in two laboratories. In order to conserve space, however, merely a single typical assay is reported here. This was performed with a pedigreed Armour's preparation of pituitary thyrotropic hormone of known concentration in order to obtain a standard nomogram for reference. Five groups of animals were used, each group representing a given amount of the hormone. In this way a representative frequency curve could be plotted for a single representative of each of three groups. Of course, each investigator should work out such a nomogram in order to standardize his entire technical procedure.

(*1*) For the zero point, the thyroids of eight control animals were studied. They were found to have a comparatively flat epithelium of uniform height. The mean acinar cell heights of these glands varied between 2.4 and 2.51, with an average of 2.46 micra. The spread of the curves was small, with a standard deviation which varied between 0.42 and 0.49.

(*2*) Eight animals were treated on five successive days with a dose of thyrotropic hormone equivalent to one-eighth of a Junkmann-Schoeller unit daily. Their thyroids responded with the slightest possible measurable hypertrophy. The mean acinar cell heights of these glands varied between 2.98 and 3.09 micra. There was a very slight increase in the spread of the frequency curves, with the standard deviation varying between 0.42 and 0.56.

(*3*) A daily dose equivalent to one-quarter of a Junkmann-Schoeller unit was given daily to ten chicks. The response was that of a greater hypertrophy than was observed in the previous group. The mean acinar cell height varied between 3.59 and 3.66 micra. The standard deviation varied between 0.47 and 0.61.

(*4*) The equivalent of one-half a Junkmann-Schoeller unit, when given in daily doses for five days, caused further hypertrophy. The incremental response at this dosage, however, was not so marked as with the previous doses. In other words, the response approaches a "ceiling" at this dose. The mean acinar cell height in this group of eight animals varied between 3.78 and 3.96 micra, with an average of 3.88 micra. The standard deviation was greater, however, varying between 0.58 and 1.19.

TABLE 45

Response of Acinar Cell Height to Increasing Dosage of Thyrotropic Hormone

Daily dose	No. of animals	Average mean cell height (μ)	Average standard deviation
Controls	8	2.46	0.45
⅛ Junkmann-Schoeller unit	8	3.03	0.49
¼ Junkmann-Schoeller unit	10	3.62	0.52
½ Junkmann-Schoeller unit	8	3.88	0.70
1 Junkmann-Schoeller unit	9	4.18	0.53

(*5*) Nine animals were treated with one Junkmann-Schoeller unit daily for five days, and responded with a measured mean acinar cell height which varied between 4.12 and 4.23 micra, with an average of 4.18 micra. The standard deviation of these curves varied between 0.45 and 0.67. Beyond this dosage, the sensitivity of the method diminishes considerably, so that assays outside of this range are not very reliable.

The increasing rise in acinar cell height is demonstrated in Table 45. It should be emphasized again that the absolute values obtained will doubtless vary from laboratory to laboratory.

The advantages of the above procedure are that (*1*) on readily available biological material (*2*) techniques which have already been tested thoroughly are combined (*3*) to give such high sensitivity that (*4*) a numerical evaluation of thyrotropic activity can be had for (*5*) concentrations of thyrotropic hormone as low as those found in human urine. Furthermore, the method can easily be calibrated against known standard material by each investigator so that his results are readily interpretable elsewhere. (Cf. Abel's clinical data, 1940.)

SOME PROPERTIES OF IODINE

A few properties of iodine are of especial interest in connection with physiological studies and are therefore enumerated.

The free halogen, I_2, has a molecular weight of 253.84. It sublimes, and its volatility may interfere with its determination. It reacts with unsaturated aliphatic compounds and with aromatic compounds, notably tyrosine, in which it displaces hydrogens in the ortho position to the hydroxyl group. It is a strong oxidizing agent; indeed, the E_0 value for the equilibrium, $[I] \rightleftharpoons I^-$, is numerically very high. More specifically, when

$$H_2 = 2\,H^+ + 2\,E^- \text{ and } E_0 = 0.000$$
$$\text{then for } 2\,I^- = I_2 + 2\,E^- \text{ the } E_0 = {}^{-}0.535\,.$$

One suspects that this property will some day be found to be related to the metabolic effects of the thyroid hormone.

The free element reacts with starch to give the characteristic starch-iodide purple color, and with dextrins to give reddish-brown erythrodextrins.

In analytical procedures the dissolved iodide ion or the free element is oxidized by free bromine to iodate.

(*1*) $I^- \rightarrow IO_3^-$
or $2\,I^- + 6\,Br_2 + 6\,H_2O = 2\,IO_3^- + 12\,Br^- + 12\,H^+$
(*2*) $I_2 + 5\,Br_2 + 6\,H_2O = 2\,HIO_3 + 10\,HBr$

If the unknown iodate to be determined is then treated with iodide, more free iodine is liberated, thus increasing the sensitivity of the titration:

(*3*) $HIO_3 + 5\,HCl + 5\,KI = 3\,I_2 + 5\,KCl + 3\,H_2O$

Final titration is often made with thiosulfate:

(*4*) $2\,S_2O_3^{=} + I_2 = S_4O_6^{=} + 2\,I^-$

Accordingly, 1 cc. of 0.005 normal thiosulfate is equivalent to 0.106 mg. of the *original* iodine.

Iodides of strong alkalies are exceeding soluble in water, and those of the alkaline earths are rather soluble. In physiological media, the iodide ion diffuses rapidly and apparently penetrates all cell membranes known thus far.

It is not known definitely that the chemical steps in the Groák method are simple reactions. Nevertheless, the following equations give an approximation of the general procedure.

(*1*) $2\,KMnO_4 + KI + H_2O \rightleftharpoons KIO_3 + 2\,MnO_2 + 2\,KOH$
(*2*) $2\,MnO_2 + 2\,HNO_2 + H_2SO_4 \rightarrow Mn(SO_4) + Mn(NO_3)_2 + 2\,H_2O$
(*3*) $CO(NH_2)_2 + 2\,HNO_2 \rightarrow CO_2 + 2\,N_2 + 3\,H_2O$

Constants and Factors

Iodine: Atomic weight 126.9, molecular weight (I_2) 253.8.

Potassium Iodide (KI): Molecular weight 166.0, iodine content 76.5 per cent.

Potassium Iodate (KIO_3): Molecular weight 214.0, iodine content 59.3 per cent.

Tyrosine ($HOC_6H_4CH_2CH(NH_2)COOH$): Molecular weight 181.1.

3:5-Diiodotyrosine ($HOC_6H_2I_2CH_2CH(NH_2)COOH$): Molecular weight 432.9, iodine content 58.6 per cent.

Thyronine ($HOC_6H_4OC_6H_4CH_2CH(NH_2)COOH$): Molecular weight 273.1.

3:5-Diiodothyronine ($HOC_6H_4OC_6H_2I_2CH_2CH(NH_2)COOH$): Molecular weight 525.0, iodine content 48.3 per cent.

Thyroxine ($HOC_6H_2I_2OC_6H_2I_2CH_2CH(NH_2)COOH$): Molecular weight 776.8, iodine content 65.4 per cent.

Iodimetry, Volumetric Equivalents

Sodium Thiosulfate: One liter of N/10 solution contains 24.85 grams when properly standardized against an appropriate oxidant, e.g., potassium iodate. One cubic centimeter of N/200 solution is equivalent to 0.635 mg. of *final* iodine, but represents 0.106 mg. of *original* iodine as liberated from iodate in the presence of potassium iodide. One cubic centimeter of N/1000 solution is equivalent to 127 micrograms of *final* iodine, but represents 21.2 micrograms of *original* iodine as liberated from iodate in the presence of potassium iodide.

Potassium Iodate: One liter of N/10 solution contains 3.567 grams. One cubic centimeter of N/10,000 solution contains 2.115 micrograms of iodine.

Iodine, N/10 Solution: One liter contains 12.692 grams of elementary iodine, first dissolved in a solution containing 36 grams (i.e., 24 or more) of potassium iodide in 100 cc. of distilled water, and subsequently diluted to exactly 1000 cc.

Solutions of Iodine (U. S. P. XI)

Compound Solution of Iodine, Liquor Iodi Compositus (Liq. Iodi Co.), "Lugol's Solution": This contains in each 100 cc. not less than 4.5 grams and not more than 5.5 grams of elementary iodine; and not less than 9.5 grams and not more than 10.5 grams of potassium iodide.

On the basis of 5.0 per cent elementary iodine and 10.0 per cent potassium iodide, 1 cc. of this solution contains approximately 50.0 mg. of elementary iodine and 126.5 mg. of total iodine.

Tincture of Iodine, Tinctura Iodi (Tr. Iodi): An aqueous-alcoholic solution of iodine and potassium iodide containing in each 100 cc. not less than 6.5 grams and not more than 7.5 grams of elementary iodine; and not less than 4.5 grams and not more than 5.5 grams of potassium iodide. The solvent contains 50 cc. of distilled water in which the potassium iodide is dissolved first, followed by the iodine, before addition of the alcohol to volume.

On the basis of 7.0 per cent of elementary iodine and 5.0 per cent potassium iodide, 1 cc. of this tincture contains 70.0 mg. of elementary iodine and 108.2 mg. of total iodine.

BIBLIOGRAPHY AND INDEX OF AUTHORS

BIBLIOGRAPHY AND INDEX OF AUTHORS

The figures in the right-hand margin indicate the pages of this book on which the authors' work is discussed. In order to avoid a too tedious cataloguing of authors, in many instances two or more papers have been listed under the senior investigator's name, with the designation "see also."

A few incidental references are listed without complete bibliographical citation because the work of the authors falls outside the scope of the present thesis. These names follow the main index.

1. Abderhalden, E., and Slavu. Über die Ausscheidung des in Form von 3,5-Dijod-l-tyrosin, Glycyl-3,5-dijod-l-tyrosin, d-Jodpropionyl-3,5-dijod-l-tyrosin und Palmityl-3,5-dijod-l-tyrosin in den Organismus des Hundes eingeführten Jods. Ztschr. f. physiol. Chem. *61*: 405. 1909. 183, 184, 202, 203

2. Abderhalden, E., and E. Wertheimer. Studien über das Verhalten von Thyroxin im tierischen Organismus. Pflüger's Arch. f. d. ges. Physiol. *221*: 82. 1929. 19

3. —— Studien über den Einfluss von Substitutionen im Thyroxinmolekül auf dessen Wirkung. Ztschr. f. d. ges. exper. Med. *63*: 557. 1928. 111, 112

4. —— Weiterer Beitrag zur Kenntnis der Wirkung des Thyroxins. Studien über Thyroxinbildung aus Thyronin (Desjodothyroxin) und Jod im tierischen Organismus. Ztschr. f. d. ges. exper. Med. *68*: 563. 1929. 111

5. Abelin, I. Über die extrathyreoidale Enstehung thyroxinartig wirkender Jodverbindungen. Klin. Wchnschr. *13*: 940. 1934. 18, 29

6. —— I. Nichtschilddrüsenstoffe mit Schilddrüsenwirkung. II. Mitteilung: Einfluss der Abbauprodukte des künstlich jodiertem Eiweisses (Homothyroxine) auf das Vogelgefieder und auf die Körpertemperatur des Meerschweinchens. Arch. f. exper. Path. u. Pharmakol. *175*: 146. 1934. III. Mitteilung: Gewinnung physiologisch aktiver Jodsubstanzen aus künstlich jodiertem Eiweiss. Ibid. *175*: 151. 1934. IV. Mitteilung: Weitere Erfahrungen über die Gewinnung schilddrüsenähnlich wirkender Substanzen aus künstlich jodiertem Eiweiss. Ibid. *181*: 250. 1936. 29, 110

7. —— Über das Vorkommen einer jodhaltigen, antithyroxinartig wirkenden Substanz in der Schilddrüse. Biochem. Ztschr. *286*: 160. 1936. 113

8. Abelin, I., and N. E. Finkelstein. Jodumsatz und Thyroxingehalt der Struma nodosa. Schweiz. med. Wchnschr. *15*: 985. 1934. 118, 124

9. Abelin, I., and A. Florin. Nichtschilddrüsenstoffe mit Schilddrüsenwirkung. Arch. f. exper. Path. u. Pharmakol. *171*: 443. 1933. 22, 29

10. Abelin, I., and C. Wegelin. Über den Einfluss des Dijodtyrosins auf die Schilddrüsenaktivität. Klin. Wchnschr. *11*: 2103. 1932. 106, 135

11. Abelson, P. An investigation of the products of the disintegration of uranium by neutrons. Physical Rev. 56: *1*. 1939. 216

12. d'Agostino, O. Nuovi elementi radioattivi artificiali. II. Gazz. chim. ital. *65*: 1071. 1935. 218

13. Allen, B. M. The relation of the pituitary and thyroid glands of Bufo and Rana to iodine and metamorphosis. Biol. Bull. Marine Biol. Lab. *36*: 405. 1919. 125

14. de Amilibia, E., M. M. Mendizábal, and J. Botella-Llusiá. Ovarialhormone und Schilddrüsenfunktion. Klin. Wchnschr. *15*: 1001. 1936. 157

15. Anderson, R. K., and H. L. Alt. The effect of thyrotropic pituitary hormone on the oxygen consumption of thyroid tissue *in vitro*. Am. J. Physiol. *119*: 67. 1937. 127

16. Anderson, A. B., C. R. Harington, and D. M. Lyon. The use of 3:5-diiodothyronine in the treatment of myxoedema. Lancet *2*: 1081. 1933. 111

17. Anselmino, K. J., and F. Hoffmann. Über den Nachweis des Schilddrüsenhormons im Schwangerenblute und über den Einfluss der gesteigerten Schilddrüsenfunktion auf Stoffwechsel, Kreislauf und Nervenerregbarkeit in der Schwangerschaft. Arch. f. Gynäk. *145*: 114. 1931. 94, 155

18. Aron, M. Particularités histologiques de la réaction de la thyroïde aux extraits de lobe antérieur d'hypophyse. Compt. rend. Soc. de biol. *103*: 145. 1930. 125, 128

19. —— L'hormone thyréo-stimulante de la préhypophyse est-elle éliminée par le rein et présente dans l'urine? Compt. rend. Soc. de biol. *114*: 20. 1933. 139, 286

20. Aron, M., and J. Benoit. Action antagoniste de la thyréostimuline préhypophysaire et de la folliculine ovarienne sur le fonctionnement thyroïden. Compt. rend. Soc. de biol. *109*: 923. 1932. 154

21. Aron, M., C. Van Caulaert, and J. Stahl. L'équilibre entre l'hormone préhypophysaire et l'hormone thyroïdienne dans le milieu intérieur, à l'état normal et à l'état pathologique. Compt. rend. Soc. de biol. *107*: 64. 1931. 132, 140

22. Aron, M., and M. Klein. Sur la présence, dans l'urine humaine, d'une substance douée de la même action sur la thyroïde que l'extrait préhypophysaire, et sur l'interprétation de la réaction de diagnostic de la grossesse. Compt. rend. Soc. de biol. *103*: 702. 1930. 133

23. Ashley, J. N., and C. R. Harington. Some derivatives of thyroxine. Biochem. J. *22*: 1436. 1928. 184

24. —— Note on the acetyl derivatives of thyroxine. Biochem. J. *23*: 1178. 1929. 51

25. Asimoff, G., and E. Estrin. Über das Schicksal des Schilddrüsenhormons im Organismus hyperthyreoidisierter Tiere. II. Mitteilung. Der Charakter der Ausscheidung des Schilddrüsenhormons nach peroraler Einführung getrockneter Schilddrüse beim Hunde. Ztschr. f. d. ges. exper. Med. *76*: 380. 1931. 185, 203, 205, 206

26. Asimoff, G., E. Estrin, and S. Miletzkaja. Über das Schicksal des Schilddrüsenhormons im Organismus hyperthyreoidisierter Tiere. IV. Mitt. Hyperthyreoidisation von Hühnern mit einmaligen Schilddrüsendosen und Bestimmung von Jod in Geweben. Ztschr. f. d. ges. exper. Med. *76*: 409. 1931. 23

27. Atnan, A., E. Fenz, and K. Uiberrak. Über das Verhalten des Blutzuckers und des Blutcholesterins nach Schilddrüsenresektion. Wien. Arch. f. inn. Med. *31*: 23. 1937. 91

28. Aub, J. C., W. Bauer, C. Heath, and M. Ropes. Studies of calcium and phosphorus metabolism. III. The effects of the thyroid hormone and thyroid disease. J. Clin. Investigation *7*: 97. 1929. 143

29. Barger, G. Die Chemie der Hormone. II. Das Thyroxin. Ergebn. d. Physiol. *27*: 780. 1928. 114

30. Barkan, G., and G. Kingisepp. Über tryptische Jodeiweissverdauung. Arch. f. exper. Path. u. Pharmakol. *160*: 610. 1931. *See also* Über die vermeintliche Jodidabspaltung aus Dijodtyrosin bei tryptischer Verdauung. Ztschr. f. physiol. Chem. *204*: 219. 1932. 52, 184

31. Barnes, B. O. The physiological activity of iodine in thyroglobulin. Am. J. Physiol. *101*: 583. 1932. 114

32. —— The excretion of iodine in experimental hyperthyroidism. Am. J. Physiol. *103*: 699. 1933. 205, 206, 212

33. Barnes, B. O., and J. G. Bueno. Studies on thyroglobulin. II. Absorption of thyroglobulin and related substances from the alimentary canal. Am. J. Physiol. *103*: 570. 1933. 82, 185

34. Barnes, B. O., A. J. Carlson, and A. M. Riskin. Studies on thyroglobulin. I. The digestibility of thyroglobulin. Am. J. Physiol. *98*: 86. 1931. 82

35. Barnes, B. O., and T. H. Chang. Further studies on the excretion of iodine in experimental hyperthyroidism. Proc. Am. Physiol. Soc. p. 3. 1933. 205

36. Bauer, H., and E. Strauss. Beiträge zur Kenntnis substituierter Proteine: Nitrierung und Jodierung des Globins. Biochem. Ztschr. *211*: 163. 1929. 7, 18, 28, 55

37. —— Beiträge zur Kenntnis substituierter Proteine. II. Mitteilung: Jodierungen des Hämoglobins und des Globins. Biochem. Ztschr. *284*: 197. 1936. 7, 55

38. —— Beiträge zur Kenntnis substituierter Proteine. III. Mitteilung: Über die Jodierung des Nitroglobins. Biochem. Ztschr. *284*: 231. 1936. 28, 45, 56

39. Baumann, E. Über das Normale Vorkommen von Jod im Thierkörper. Ztschr. f. physiol. Chem. *21*: 319 (especially p. 322). 1895–96. *See also* Über das normale Vorkommen des Jods im Thierkörper. III. Mitteilung. Der Jodgehalt der Schilddrüsen von Menschen und Thieren. Ibid. *22*: 1. 1896–97. 4, 11, 27, 31

40. —— Über die Jodverbindung der Schilddrüse. München. med. Wchnschr. *43*: 398. 1896. 4, 12, 184

41. Baumann, E. J., and N. Metzger. On the amount of iodine in blood. J. Biol. Chem. *121*: 231. 1937. *See also* Iodine in pituitary and some other tissues. Ibid. *127*: 111. 1939. 19, 138

42. Beaver, D. C., and J. de J. Pemberton. The pathologic anatomy of the liver in exophthalmic goiter. Ann. Int. Med. *7*: 687. 1933. 210

43. Bergman, A. J., and C. W. Turner. A comparison of the guinea pig and chick thyroid in the assay of the thyrotropic hormone. Endocrinology *24*: 656. 1939. 287

44. Bergmann, M. Proteins and proteolytic enzymes. Harvey Lectures *31*: 37. 1935–36. 37, 41

45. Bier, A., and W. Roman. Das Wesen der postoperativen Reaktion beim Morbus Basedowii nach Thyreoidektomie. Ztschr. f. klin. Med. *118*: 15. 1931. 73, 87, 91

46. Blau, N. F. The determination of thyroxine in the thyroid gland. J. Biol. Chem. *102*: 269. 1933. 27, 284

47. Blum, F. Zur Chemie und Physiologie der Jodsubstanz der Schilddrüse. Pflüger's Arch. f. d. ges. Physiol. *77*: 70. 1899. *See also* Über die antithyreoidalen Eigenschaften des Blutes und das zugrundeliegende Katechin. Schweiz. med. Wchnschr. *63*: 777. 1933. 93

48. Blum, F., and E. Strauss. Mitteilungen aus dem Gebiete der Eiweisschemie. I. Über Jodbindungsfähigkeit und Konstitution der Proteine. Ztschr. f. physiol. Chem. *112*: 111. 1921. 28, 45, 54

49. —— Mitteilungen aus dem Gebiet der Eiweisschemie. III. Über Jodierung von Proteinen mit Jodstickstoff. Ztschr. f. physiol. Chem. *127*: 199. 1923. 45

50. Blum, F., and W. Vaubel. Ueber Halogeneiweissderivate. J. f. präkt. Chem. *56*: 393. 1897. 50, 53

51. —— Ueber Halogeneiweissderivate. J. f. präkt. Chem., neue Folge, *57*: 365. 1898. 45

52. Böe, J., and A. W. Elmer. Über den Einfluss der intravenösen Thyroxininjektion auf den Blutjodspiegel und die Harnjodausscheidung bei Menschen. Biochem. Ztschr. *240*: 187. 1931. 82, 184, 203

53. Böhm, R., and F. Berg. Arbeiten aus dem pharmakologischen Institut der Universität Dorpat. 12. Beiträge zur Pharmakologie des Jod. Arch. f. exper. Path. u. Pharmakol. *5*: 329. 1876. 27

54. Boothby, W. M. The total and basal metabolism in exophthalmic goiter. Minnesota Med. *7*: 91. 1924. 237

55. —— Disease of the thyroid gland. Arch. Int. Med. *56*: 136. 1935. 258

56. Boothby, W. M., and E. J. Baldes. Some quantitative relationships of thyroxin calculated from its calorigenic action. J. Pharmacol. & Exper. Therap. *25*: 139. 1925. 108, 120

57. —— Activation and decay curves of thyroxin. Proc. Staff Meet., Mayo Clin. *1*: 166. 1926. 255

58. Boothby, W. M., O. B. Buckley, and C. M. Wilhelmj. The influence of work on the calorigenic action of thyroxine. J. Physiol. *74*: 376. 1932. 208

59. Brain, W. R. Exophthalmic ophthalmoplegia. Quart J. Med. *7*: 293. 1938. 260

60. Bram, I. Exophthalmic goiter in patients past age fifty: comments based on a series of 322 cases. Pennsylvania M. J. *42*: 117. 1938. *See also* Exophthalmic Goiter and Its Medical Treatment. St. Louis, C. V. Mosby Company. 1936. 147, 259, 261

61. Brand, E., and B. Kassell. The photometric determination of tryptophane, tyrosine, diiodotyrosine and thyroxine. J. Biol. Chem. *131*: 489. 1939. 25

62. Brand, E., B. Kassell, and M. Heidelberger. On the structure of thyroglobulin. J. Biol. Chem. *128*: xi. 1939. 37

63. Bratton, A. C., and J. F. McClendon. Determination of iodine in drinking water, urine, and substances containing only about 1000 times as much organic matter as iodine. Indust. & Engin. Chem., Analytical Ed. *10*: 600. 1938. 271

64. Breitner, B. Die praktische Verwertbarkeit unseres heutigen Wissens über den Jodstoffwechsel der Schilddrüse. Wien. klin. Wchnschr. *45*: 641. 1932. 89

65. Brooks, C. McC. A study of the mechanism whereby coitus excites the ovulation-producing activity of the rabbit's pituitary. Am. J. Physiol. *121*: 157. 1938. 173

66. Brooks, C. McC., and E. F. Lambert. The effect of hypophysial stalk transection on the gonadotropic functions of the rabbit's hypophysis. Am. J. Physiol. *128*: 57. 1939–40. 172, 173

67. Brown, A. W., I. P. Bronstein, and R. Kaines. Hypothyroidism and cretinism in childhood. VI. Influence of thyroid therapy on mental growth. Am. J. Dis. Child. *57*: 517. 1939. 252

68. Bubnow, N. A. Beitrag zu der Untersuchung der chemischen Bestandtheile der Schilddrüse des Menschen und des Rindes. Ztschr. f. physiol. Chem. *8*: 1. 1883–84. 4, 32

69. Bürger, M., and W. Möbius. Der Jod- und Cholesteringehalt des Blutes in seinen Beziehungen zur essentiellen Hypertonie. Klin. Wchnschr. *13*: 1349. 1934. 72

70. Cahane, M., and T. Cahane. Sur l'existence des centres nerveux infundibulaires réglant la fonction du corps thyroïde. Acta med. Scandinav. *94*: 320. 1938. 162

71. Cameron, A. J. D. Thyroid dysfunction: its treatment by tyronorman and diet. M. Press *190*: 200. 1935. 93

72. Cannon, W. B., C. A. L. Binger, and R. Fitz. Experimental hyperthyroidism. Am. J. Physiol. *36*: 363. 1915. 166

73. Cannon, W. B., and R. Fitz. Further observations on overactivity of the cervical sympathetic. Am. J. Physiol. *40*: 126. 1916. 166

74. Cannon, W. B., and A. Rosenblueth. Studies on conditions of activity in endocrine organs. XXIX. Sympathin E and sympathin I. Am. J. Physiol. *104*: 557. 1933. 171

75. Canzanelli, A., C. R. Harington, and S. S. Randall. The configurative relationship of thyroxine and tyrosine. Biochem. J. *28*: 68. 1934. 110

76. Canzanelli, A., and D. Rapport. The effect of thyrotropic hormone, alone and with other substances, on the *in vitro* oxygen consumption of thyroid and liver. Endocrinology *22*: 73. 1938. 127

77. Carson, D. A., and W. Dock. The effect of iodin upon experimental hyperthyroidism in man. Am. J. M. Sc. *176*: 701. 1928. 122

78. Carter, G. S. Iodine compounds and fertilisation. VI. Physiological properties of extracts of the ovaries and testes of Echinus esculentus. J. Exper. Biol. *9*: 253. 1932. 21, 22, 148, 150

79. Castleman, B., and S. Hertz. Pituitary fibrosis with myxedema. Arch. Path. *27*: 69. 1939. 59, 269

80. Cavett, J. W. Thyroglobulin studies. II. The van Slyke nitrogen distribution and tyrosine and tryptophane analyses for normal and goitrous human thyroglobulin. J. Biol. Chem. *114*: 65. 1936. 43, 124

81. Cavett, J. W., C. O. Rice, and J. F. McClendon. Thyroglobulin studies. I. Thyroxine and iodine content of normal and goitrous human thyroglobulin. J. Biol. Chem. *110*: 673. 1935. 8, 33, 42, 119, 124

82. —— Is the high basal metabolic rate in "hyperthyroidism" due to thyroxine? Science *80*: 19. 1934. 118

83. Chatin, A. Un fait dans la question du goître et du crétinisme. Compt. rend. Acad. d. sc. *36*: 652. 1853. 4, 188

84. Chesney, A. M., T. A. Clawson, and B. Webster. Endemic goitre in rabbits. I. Incidence and characteristics. Bull. Johns Hopkins Hosp. *43*: 261. 1928. 191, 254

85. Chidester, F. E. The relation of iodin to the effectiveness of endocrine extracts. Arch. internat. de pharmacodyn. et de thérap. *48*: 354. 1934. 6

86. Chouke, K. S., H. Friedman, and L. Loeb. Proliferative activity of the thyroid gland of the female guinea pig during the sexual cycle. Anat. Rec. *63*: 131. 1935. 154, 156, 157

87. Closs, K. The iodine content of commercial desiccated anterior pituitary preparations. J. Pharmacol. & Exper. Therap. *43*: 131. 1931. 19, 138

88. Closs, K., L. Loeb, and E. M. Mackay. The effect of an acid extract of the anterior pituitary on the iodine concentration of the blood and thyroid gland. J. Biol. Chem. *96*: 585. 1932. 129, 130

89. Clutton, R. F., C. R. Harington, and M. E. Yuill. Studies in synthetic immunochemistry. III. Preparation and antigenic properties of thyroxyl derivatives of proteins, and physiological effects of their antisera. Biochem. J. *32*: 1119. 1938. 118

90. Cohen, R. A., and R. W. Gerard. Hyperthyroidism and brain oxidations. J. Cell. & Comp. Physiol. *10*: 223. 1937. 160

91. Cohn, B. N. E. Absorption of compound solution of iodine from the gastro-intestinal tract with special reference to the absorption of free iodine. Arch. Int. Med. *49*: 950. 1932. 100, 182

92. Cohn, E. J. Die physikalische Chemie der Eiweisskörper. Ergebn. d. Physiol. *33*: 781. 1931. 28, 46

93. Cohn, E. J., W. T. Salter, and R. J. Ferry. The amphoteric properties of globin and iodized globin. J. Biol. Chem., Soc. Proc. XXXII, *123*: xxiv. 1938. 29, 49, 110

94. Coindet, J. R. Découverte d'un nouveau remède contre le goître. Ann. de chim. et phys. *15*: 49. 1820. 27

95. Cole, V. V., and G. M. Curtis. Cyclic variations in urinary excretion of iodine in women. Proc. Soc. Exper. Biol. & Med. *31*: 29. 1933. 151, 153

96. —— Human iodine balance. J. Nutrition *10*: 493. 1935. *See also* Iodine metabolism of the adult rat in relation to trauma, thyroid activity and diet. J. Pharmacol. & Exper. Therap. *56*: 351. 1936. 186, 188, 193, 194, 208, 210, 227

97. Collip, J. B., and E. M. Anderson. The production of serum inhibitory to the thyrotropic hormone. Lancet *1*: 76. 1934. 93, 129

98. —— Studies on the thyrotropic hormone of anterior pituitary. J.A.M.A. *104*: 965. 1935. 126

99. Collip, J. B., H. Selye, and D. L. Thomson. The antihormones. Biol. Rev. *15*: 1. 1940. 94, 129

100. Condorelli, L. Ricerche sperimentali sul significato e sul meccanismo d'azione della diiodotirosina. VI. Considerazioni sui risultati di un primo gruppo di ricerche sperimentali. Riv. di pat. sper. *8*: 197. 1937. 110

101. Cope, C. L.: discussion of A. Loeser. Hyperthyroidism and the thyrotropic hormone of the pituitary. Brit. M. J. *1*: 1276. 1937. 140

102. —— The anterior pituitary lobe in Graves' disease and in myxoedema. Quart. J. Med. 7: 151. 1938. 133, 139

103. —— The young chick as test for the thyrotropic hormone. J. Physiol. *94*: 358. 1938. 287

104. Courrier, R., and M. Aron. Sur le passage de l'hormone thyroïdienne de la mère au foetus à travers le placenta. Compt. rend. Soc. de biol. *100*: 839. 1929. 194

105. Courth, H. Der Jodgehalt der Pferdeschilddrüsen. Biochem. Ztschr. *232*: 310. 1931. 105

106. —— Untersuchungen über den Jodstoffwechsel normaler und thyreoidektomierter Ziegen bei Jodalkali- und Pflanzenjodfütterung. Biochem. Ztschr. *238*: 162. 1931. 70, 193, 194, 206

107. Courtois, M. B. Découverte d'une substance nouvelle dans le Vareck. Ann. de chim. *88*: 304. 1813. 4, 268

108. Cramer, W., and E. S. Horning. The prevention of spontaneous mammary cancer in mice by the thyrotropic hormone of the pituitary gland. Lancet *1*: 72. 1938. 156

109. Crile, G. Indications for the use of iodine in the treatment of hyperthyroidism. M. Clin. North America *22*: 489. 1938. *See also* G. Crile, et al. Diagnosis and Treatment of Diseases of the Thyroid Gland. Philadelphia and London, W. B. Saunders Company. 1932. Cf. Chap. XXXIV, p. 419. 143

110. Cucco, G. P. Contributo alla conoscenza sul contenuto iodico del sangue. Fisiol. e med. *3*: 117. 1932. 93, 152

111. Curtis, G. M., and L. E. Barron. The urinary loss of iodine following total thyroidectomy. Surgery *1*: 92. 1937. 194

112. Curtis, G. M., V. V. Cole, and F. J. Phillips. The blood iodine in thyroid disease. West. J. Surg. *42*: 435. 1934. 83, 85, 250

113. Curtis, G. M., C. B. Davis, and F. J. Phillips. Significance of the iodine content of human blood. J.A.M.A. *101*: 901. 1933. 94

114. Curtis, G. M., and F. J. Phillips. The urinary excretion of iodine. I. The loss of iodine in the urine following thyroidectomy. J. Clin. Investigation *13*: 777. 1934. 91, 194, 206, 208, 210

115. Curtis, G. M., and I. D. Puppel. Increased urinary excretion of iodine in hyperthyroidism. Arch. Int. Med. *60*: 498. 1937. 208

116. Curtis, G. M., I. D. Puppel, V. V. Cole, and N. L. Matthews. The normal urinary iodine of man. J. Lab. & Clin. Med. *22*: 1014. 1937. 186, 193

117. Dale, H. H. Transmission of nervous effects by acetylcholine. The Harvey Lectures. Baltimore, Williams and Wilkins Company. Series XXXII. 1937. 171

118. Dale, H. H., and W. Feldberg. The chemical transmission of secretory impulses to the sweat glands of the cat. J. Physiol. *82*: 121. 1934. 171

119. Danilewski, quoted by V. Henriques, and I. K. Gjaldbäk. Untersuchungen über die Plasteinbildung. Ztschr. f. physiol. Chem. *71*: 485. 1911. 62

120. Dautrebande, L. Physiopathologie de la thyroïde. Congrès français de médecine *21*: 51. Session, Liége. 1930. 16

121. Davis, C. B., G. M. Curtis, and V. V. Cole. Blood iodine studies. II. Normal iodine content of human blood. J. Lab. & Clin. Med. *19*: 818. 1934. 79

122. Dobrzaniecki, W., and M. Aron. Influence de l'excitation du sympathique cervical sur le fonctionnement thyroïdien. Compt. rend. Soc. de biol. *104*: 1321. 1930. 166

123. Dodds, E. C., W. Lawson, and J. D. Robertson. Variations in the iodine content of the blood in hyperthyroidism and non-toxic goitre. Lancet *2*: 608. 1932. 87

124. Dorff, G. B. Sporadic cretinism in one of twins. Report of cases with roentgen demonstration of osseous changes that occurred in utero. Am. J. Dis. Child. *48*: 1316. 1934. 71

125. Drechsel, E. Beiträge zur Chemie einiger Seethiere. Ztschr. f. Biol. *33*: 85. 1896. 7, 27, 28

126. —— Die wirksame Substanz der Schilddrüse. Zentralbl. f. Physiol. *9*: 705. 1895–96. 50

127. Drennan, A. M., J. Malcolm, and G. A. Cox. Studies on rat thyroids. Brit. J. Exper. Path. *12*: 430. 1931. 182

128. Dresel, K., and M. Goldner. Ein neuer biologischer Test für Schilddrüsenstoffe. Ber. ü. d. ges. Physiol. *53*: 387. 1930. 212

129. DuBridge, L. A. Theory of the energy distribution of photoelectrons. Physical Rev. *43*: 727. 1933. *See also* Rev. Scient. Instruments *4*: 532. 1933. 218

130. Eason, J. Primary toxic goitre. Edinburgh M. J. *45*: 529. 1938. 265

131. Eggenberger, H. Kropf und Kretinismus in Handbuch der inneren Sekretion. Edited by M. Hirsch. Leipzig, c. Kabitzsch. *3*: 684. 1928. 181, 186, 192

132. —— Jodthyreotoxicosen und ihre Verhütung durch biologische Joddosen. Tr. Third Internat. Goiter Conf. Pub. by West. J. Surg., Portland, Oregon. 1938. p. 68. 253

133. Eisler, B., and A. Schittenhelm. Über die Wirkung des Thyroxins auf den Blutjodgehalt des Myxödematösen. Ztschr. f. d. ges. exper. Med. 68: 487. 1929. 83, 84

134. Eitel, H., H. A. Krebs, and A. Loeser. Hypophysenvorderlappen und Schilddrüse. Die Wirkung der thyreotropen Substanz des Hypophysenvorderlappens auf die Schilddrüse *in vitro*. Klin. Wchnschr. *12*: 615. 1933. 129, 166

135. Elmer, A. W. Beiträge zum Jodstoffwechsel im menschlichen Organismus. I. Der Blutjodspiegel und die Harnjodausscheidung nach einmaliger intravenöser Injektion von anorganischem Jod bei Menschen. Fälle mit normaler Schilddrüse (Euthyreosen). Deutsches Arch. f. klin. Med. *174*: 390. 1933. 81

136. —— Beiträge zum Jodstoffwechsel im menschlichen Organismus. II. Mitt. Der Blutjodspiegel und die Harnjodausscheidung nach einmaliger intravenöser Injektion vom anorganischem Jod bei Hypothyreosen. Deutsches Arch. f. klin. Med. *174*: 449. 1933. 124

137. —— Iodine tolerance test for thyroid insufficiency. Endocrinology *18*: 487. 1934. 84, 106, 207, 266

138. —— La physio-pathologie du métabolisme de l'iode et son rapport avec la fonction de la glande thyroïde. Paris méd. *101*: 433. 1936. 6, 22, 24, 25, 101, 137

139. —— Iodine Metabolism and Thyroid Function. London, Oxford Univ. Press. 1938. 4, 19, 22, 70, 71, 81, 83, 84, 94, 101, 108, 123–125, 143, 184, 188, 190, 192–194, 202, 207, 210

140. —— Personal communication. 1939. *See also* Di-iodotyrosine and thyroid function. Quart. J. Exper. Physiol. *24*: 95. 1935. 140, 183

141. Elmer, A. W., B. Giedosz, and M. Scheps. Sur l'action cortico-stimulante et médullo-stimulante du lobe antérieur de l'hypophyse. Compt. rend. Soc. de biol. *125*: 1082. 1937. 143

142. Elmer, A. W., B. Giedosz, and M. Scheps. L'action de la vitamine B_1 dans l'hyperthyréose expérimentale. Compt. rend. Soc. de biol. *126*: 1037. 1937. 103

143. —— Histology of diabetes induced in dogs by injections of anterior-pituitary extracts. Lancet *1*: 1307. 1938. 143

144. —— Action des vitamines A, C, D sur la thyroïde normale et sur la thyroïde hyperactivée par la thyréostimuline préhypophysaire; effet inhibiteur des vitamines A et de l'acide ascorbique dans l'hyperthyréose expérimentale. Compt. rend. Soc. de biol. *120*: 560. 1935. 103

145. Elmer, A. W., and J. Lankosz. Relation of the pituitary to thyroid disturbances. Lecture before the Harvard Med. Soc., Boston. 1939. 85, 127, 129, 134

146. Elmer, A. W., and Z. Luczynski. L'excrétion d'iode par la bile à jeun et après le repas. Compt. rend. Soc. de biol. *114*: 1340. 1933. 193

147. —— Sur l'élimination par la bile de la thyroxine administrée par voie entérale. Compt. rend. Soc. de biol. *115*: 647. 1933. 193

148. —— Rôle du foie dans la régulation du taux de l'iode dans le sang. Compt. rend. Soc. de biol. *115*: 1717. 1934. 204, 205

149. Elmer, A. W., Z. Luczynski, and M. Scheps. Sur la thyroxinémie normale chez l'homme et chez l'animal. Compt. rend. Soc. de biol. *115*: 1714. 1934. 18, 76

150. Elmer, A. W., and Wl. Rychlik. Récherches comparatives sur l'excrétion d'iode par l'urine, après ingestion d'iode minéral, de thyroxine et de diiodotyrosine. Compt. rend. Soc. de biol. *115*: 1719. 1934. 183, 184, 202, 203

151. —— L'élimination de l'iode par le colostrum et par le lait pendant l'accouchement chez la femme. Compt. rend. Soc. de biol. *117*: 530. 1934. 71, 186, 191, 194

152. Elmer, A. W., Wl. Rychlik, and M. Scheps. L'hyperthyroxinémie dans la thyréotoxicose. Compt. rend. Soc. de biol. *117*: 533. 1934. 88

153. Elmer, A. W., and M. Scheps. Sur la forme d'iode éliminé par l'urine, après administration, par voie buccale, de thyroxine chez l'homme. Compt. rend. Soc. de biol. *114*: 350. 1933. 82

154. —— La thyroxine est-elle éliminée par les reins chez l'homme normal et chez les Basedowiens? Compt. rend. Soc. de biol. *115*: 968. 1934. 193, 208

155. —— The iodine content of blood and the urine and the basal metabolic rate; their value in the diagnostic of the function of the thyroid gland. Acta med. Scandinav. *82*: 126. 1934. 83, 87, 94, 206

156. —— Sur la teneur en thyroxine et en diiodotyrosine de la thyroïde des nouveau-nés et des foetus. Compt. rend. Soc. de biol. *118*: 1370. 1935. 14

157. —— Distribution de l'iode dans les substances corticale et médullaire des capsules surrénales. Compt. rend. Soc. de biol. *118*: 1374. 1935. 6, 20

158. EMERSON, K., JR., AND W. C. CUTTING. Urinary thyrotropic hormone. Endocrinology *23*: 439. 1938. 139

159. EPPINGER, E. C., AND W. T. SALTER. The daily requirement in human hypothyroidism of purified human thyroid hormone at various metabolic levels. A comparison between spontaneous myxedema and cachexia strumipriva. Am. J. M. Sc. *190*: 649. 1935. 84, 89, 108

160. ETTISCH, G., AND W. EWIG. Zur Technik der Elektrodialyse, insbesondere der Mikroelektrodialyse. Biochem. Ztschr. *200*: 250. 1928. 73

161. EUFINGER, H., AND W. SCHULTE. Der Nachweis des organisch gebundenen Jods im Blute und seine Bedeutung in der normalen und toxischen Schwangerschaft. Arch. f. Gynäk. *152*: 479. 1933. 73

162. EVANS, H. E., M. E. SIMPSON, AND R. I. PENCHARZ. Relation between the growth promoting effects of the pituitary and the thyroid hormone. Endocrinology *24*: 875. 1939. 128, 144

163. EVANS, R. J., AND P. H. PHILLIPS. Fluorine content of thyroid gland in cases of hyperthyroidism. J.A.M.A. *111*: 300. 1938. 192

164. FALTA, W., AND E. FENZ. Bemerkungen zum Problem Schilddrüse-Zwischenhirn. Klin. Wchnschr. *17*: 148. 1938. 85

165. FASHENA, G. J. A study of the blood iodine in childhood. J. Clin. Investigation *17*: 179. 1938. 79

166. FASHENA, G. J., AND V. TREVORROW. A note on the determination of iodine in biological material. J. Biol. Chem. *114*: 351. 1936. 271, 276

167. FELLENBERG, TH. VON. Untersuchungen über den Jodstoffwechsel. II. Biochem. Ztschr. *174*: 341. 1926. *See also* Versuche über die Jodspeicherung in den einzelnen Organen. Ibid. p. 355. *See also* Über jodiertes Kochsalz. III. Ibid. p. 364. 4, 20, 22, 23, 81, 101, 181

168. —— Das Vorkommen, der Kreislauf und der Stoffwechsel des Jods. Ergebn. d. Physiol. *25*: 176. 1926. 4, 23, 70, 71, 187–189, 194, 201, 202, 207, 212

169. —— Untersuchungen über den Jodstoffwechsel. III. Biochem. Ztschr. *184*: 85. 1927. 188

170. FELLINGER, K. Klinische und experimentelle Untersuchungen über das Verhalten und die Bedeutung des thyreotropen Hormons im Blute. Wien. Arch. f. inn. Med. *29*: 375. 1936. 139

171. FELLINGER, K., AND O. HOCHSTÄDT. Über die Beeinflussung des Reid Huntschen Versuches durch antithyreoidale Schutzstoffe. Klin. Wchnschr. *14*: 1250. 1935. 94

172. FENZ, E. Über die Wirkung der Zwischenhirnnarkose bei Morbus Basedow. Wien. Arch. f. inn. Med. *30*: 15. 1937. 263

173. FENZ, E., AND K. UIBERRAK. Über die Wirkung der Zwischenhirnnarkose auf das Blutjod bei Morbus Basedow. Wien. Arch. f. inn. Med. *30*: 135. 1937. 85, 93, 176

174. FITZ, R., AND R. S. HUNT. Experiences with an iodine tolerance test in "hypothyroid" patients. Tr. A. Am. Physicians *52*: 24. 1937. 81, 106

175. Foot, N. C., L. E. Baker, and A. Carrel. The behavior of abnormal human thyroid tissue cultivated in the Lindbergh apparatus. J. Exper. Med. *70*: 39. 1939. 103, 129

176. Foster, G. L. A comparison of the effects of administration of iodine and diiodotyrosine on the iodine and thyroxine content of the thyroid. J. Biol. Chem. *104*: 497. 1934. 122

177. —— The isolation of 3,5-diiodotyrosine from the thyroid. J. Biol. Chem. *83*: 345. 1929. *See also* Foster, G. L., and A. B. Gutman. On the fate of diiodotyrosine in the animal organism. Ibid. *87*: 289. 1930. 51, 183, 202, 203

178. Foster, G. L., A. B. Gutman, and E. B. Gutman. Total and thyroxine iodine content of thyroid gland after injection of saline anterior pituitary extracts. Proc. Soc. Exper. Biol. & Med. *30*: 1028. 1933. 119

179. Foster, G. L., W. W. Palmer, and J. P. Leland. A comparison of the calorigenic potencies of l-thyroxine, dl-thyroxine, and thyroid gland. J. Biol. Chem. *115*: 467. 1936. 51, 111, 116, 284

180. Foster, G. L., and P. E. Smith. Hypophysectomy and replacement therapy in relation to basal metabolism and specific dynamic action in the rat. J.A.M.A. *87*: 2151. 1926. 125

181. Foster, R. C., and M. J. Thornton. Thyroid in the treatment of menstrual irregularities. Endocrinology *24*: 383. 1939. 157

182. Franck, S. Histophysiologie de la préhypophyse. Préhypophyse et glande thyroïde soumises à l'action de la folliculine. Compt. rend. Soc. de biol. *125*: 573. 1937. 157

183. Franke, M., and L. Ptaszek. Taux de l'iode dans le sang après la castration chez les chiennes. Compt. rend. Soc. de biol. *114*: 1129. 1933. 153, 155, 205

184. Freud, J., and E. Laqueur. Comparison between the effect of thyroxin and thyroid extracts upon the metabolism of rats. Acta brev. Neerland. *6*: 104. 1936. 116

185. Frey, E. Das Gesetz der Abwanderung intravenös injizierten Stoffes aus dem Blute und seine Verteilung auf Blut und Gewebe. Pflüger's Arch. f. d. ges. Physiol. *177*: 110. 1919. 202

186. Friedgood, H. B. Experimental exophthalmos and hyperthyroidism in guinea-pigs. Clinical course and pathology. Bull. Johns Hopkins Hosp. *54*: 48. 1934. 134

187. —— Cortico-adrenal and neural effects on gonadotropic activity of the pituitary. Science *86*: 84. 1937. *See also* H. B. Friedgood, and S. Bevin. Relation of the cervical sympathetics to pseudopregnancy in the rat. Am. J. Physiol. (Proc.) *123*: 71. 1938. 175

188. Friedgood, H. B., and W. B. Cannon. Studies on the sympathetic nervous control of the anterior hypophysis. Am. J. Physiol. *116*: 54. 1936. *See also* Autonomic control of thyroid secretion. Endocrinology *26*: 142. 1940. 166, 167

189. Friedgood, H. B., and G. Pincus. Studies on conditions of activity in endocrine organs. XXX. The nervous control of the anterior hypophysis as indicated by maturation of ova and ovulation after stimulation of cervical sympathetics. Endocrinology *19*: 710. 1935. 145

190. Fukushima, T. Zur Frage der Funktion der Schilddrüse in Bezug auf den Jodstoffwechsel. I. Über den Jodgehalt der Schilddrüsen erwachsener Japaner. II. Das Schicksal des zugeführten Jods in normalen und thyreopriven Ratten. Mitt. a. d. med. Fak. d. k. Univ. z. Tokyo *30*: 557; 571. 1922–23. 101

191. Gaddum, J. H. Quantitative observations on thyroxine and allied substances. I. The use of tadpoles. J. Physiol. *64*: 246. 1927. II. Effects on the oxygen consumption of rats. Ibid. *68*: 383. 1929–30. 111, 112, 184

192. Garnier, M., and R. Huguenin. Glande Thyroïde. Traité de physiologie normale et pathologique. IV. Les sécrétions internes. Edited by G.–H. Roger. Paris, Masson et Cie. 1928. 141

193. Gersh, I. "Glandular" cells in the pars nervosa and stalk of the hypophysis. Proc. Soc. Exper. Biol. & Med. *37*: 395. 1937. 172, 174

194. Gessler, C. Influence of folliculin on the basal metabolic rate. Arch. internat. de pharmacodyn. et de thérap. *54*: 263. 1936. 158

195. —— Activité antithyroïdienne de la folliculine; rôle de l'hypophyse. Arch. internat. de pharmacodyn. et de thérap. *55*: 267. 1937. 154

196. Giedosz, B. Hiperluteinizacja doświadczalna. Polska gaz. lek. *14*: 733. 1935. 156

197. Ginsburg, S. Postoperative progressive exophthalmos with low basal metabolic rate. Ann. Int. Med. *13*: 424. 1939. 260

198. Gley, E. La thyroïde, les progrès de l'endocrinologie et la biologie générale. Endokrinologie *5*: 73. 1929. *See also* E. Gley, and P. Bourcet. Présence de l'iode dans le sang. Compt. rend. Acad. d. sc. *130*: 1721. 1900. 95

199. Glimm, E., and J. Isenbruch. Über die Bestimmung kleinster Jodmengen. Biochem. Ztschr. *207*: 368. 1929. 15, 72

200. Goudsmit, S. Radioactivity in biological experiments. Science *90*: 615. 1939. 213, 232

201. Grab, W. Die funktionelle Bedeutung der Strukturelemente der Schilddrüse. Klin. Wchnschr. *12*: 1102. 1933. *See also* Die Zusammenarbeit der Zellen und des Kolloids bei der inkretorischen Tätigkeit der Schilddrüse. Ibid. p. 1637. 12, 25, 119

202. Graham, D. The use and abuse of iodine in the treatment of goitre. Canad. M. A. J. *14*: 821. 1924. 253

203. Graham, E. A., and W. H. Cole. Roentgenologic examination of the gall bladder. Preliminary report of a new method utilizing the intravenous injection of tetrabromphenolphthalein. J.A.M.A. *82*: 613. 1924. 66, 205

204. Graham, W. R., Jr. The effect of thyroidectomy and thyroid feeding on the milk secretion and milk fat production of cows. J. Nutrition *7*: 407. 1934. 157

205. Grauer, R. C., and E. Saier. A comparison of the distilling and dry ashing methods for the determination of blood iodine. Endocrinology *24*: 553. 1939. 270

206. Groák, B. Mikrogesamtjodbestimmung und Jodbestimmung in organischen Säuren. Biochem. Ztschr. *175*: 455. 1926. 271, 273

207. —— Permanganatoxidation in der Jodmikromethodik. Biochem. Ztschr. *270*: 291. 1934. 65, 275, 280

208. Gudernatsch, J. F. Feeding experiments of tadpoles. I. The influence of specific organs given as food on growth and differentiation; a contribution to the knowledge of organs with internal secretion. Arch. f. Entwcklngsmechn. d. Organ. *35*: 457. 1912–13. 19

209. Guernsey, P., quoted by W. Engelbach. Endocrine Medicine. *1*: 21–23. 1932. Baltimore, Charles C. Thomas. 8, 144

210. Gutman, A. B., E. N. Benedict, B. Baxter, and W. W. Palmer. The effect of administration of iodine on the total iodine, inorganic iodine, and thyroxine content of the pathological thyroid gland. Tr. Am. A. Study Goiter p. 31. 1932. Also condensed in J. Biol. Chem. *97*: 303. 1932. 7, 16, 25, 101, 106, 120, 123, 124, 231

211. Gutman, A. B., L. W. Sloan, E. B. Gutman, and W. W. Palmer. The rôle of diiodotyrosine in hyperthyroidism. A comparison of the therapeutic effect of diiodotyrosine with inorganic iodine. J.A.M.A. *101*: 256. 1933. 112, 122

212. Gutzeit, K., and G. W. Parade. Blutjodstudien. Mitt. I–VI. Ztschr. f. klin. Med. *133*: 503–551. 1937–38. 79, 83, 87–90, 93

213. —— Blutjodstudien: der Einfluss körperlicher Anstrengung auf die Blutjodverhältnisse beim Normalen und Hyperthyreotiker. Ztschr. f. klin. Med. *135*: 158. 1938. *See also* Blutjodstudien. Mitt. VIII–XI. Ibid. *135*: 166–186. 1939. 79, 88–91, 93, 174

214. Hahn, A., and A. Schürmeyer. Über den Jodgehald des Liquor cerebrospinalis. Klin. Wchnschr. *11*: 421. 1932. 67

215. Hair, G. W. The nerve supply of the hypophysis of the cat. Anat. Rec. *71*: 141. 1938. 173

216. Hamilton, J. G. Personal communication, May 3, 1939. 229, 234

217. —— The rates of absorption of the radioactive isotopes of sodium, potassium, chlorine, bromine, and iodine in normal human subjects. Am. J. Physiol. *124*: 667. 1938. 182, 213

218. Hamilton, J. G., and M. H. Soley. Studies in iodine metabolism by the use of a new radioactive isotope of iodine. Am. J. Physiol. *127*: 557. 1939. 212, 215, 217, 218

219. Hamilton, J. G., and R. S. Stone. The intravenous and intraduodenal administration of radio-sodium. Radiology *28*: 178. 1937. 213

220. Harington, C. R. The Thyroid Gland, Its Chemistry and Physiology. London, Oxford Univ. Press. 1933. 4, 7, 100, 112, 118, 146

221. —— Biochemical basis of thyroid function. Lancet *1*: 1199; 1261. 1935. 18, 115

222. ——The biochemistry of the thyroid. Ergebn. d. Physiol. *37*: 210. 1935. 122

223. Harington, C. R., and G. Barger. Chemistry of thyroxine. III. Constitution and synthesis of thyroxine. Biochem. J. *21*: 169. 1927. 4, 52, 112

224. Harington, C. R., and W. McCartney. Synthesis of an isomeride of thyroxine and of related compounds. J. Chem. Soc. *1929*: 892. 1929. 111

225. Harington, C. R., and R. V. Pitt Rivers. Preparation of thyroxine from casein treated with iodine. Nature *144*: 205. 1939. 50, 51, 60

226. Harington, C. R., and S. S. Randall. Observations on the iodine-containing compounds of the thyroid gland. Isolation of dl-3:5-diiodotyrosine. Biochem. J. *23*: 373. 1929. 4, 28, 30

227. ——The chemical assay of thyroid gland. Quart. J. Pharm. & Pharmacol. *2*: 501. 1929. 24, 113, 150, 230, 284

228. ——The isolation of d-3:5 diiodotyrosine from the thyroid gland by the action of proteolytic enzymes. Biochem. J. *25*: 1032. 1931. 13, 50

229. Harington, C. R., and W. T. Salter. The isolation of l-thyroxine from the thyroid gland by the action of proteolytic enzymes. Biochem. J. *24*: 456. 1930. 4, 12, 13, 18, 51, 59, 61, 111, 114, 184, 185

230. Harnack, E. Ueber das Jodospongin, die jodhaltige eiweissartige Substanz aus dem Badeschwamm. Ztschr. f. physiol. Chem. *24*: 412. 1898. 31

231. Haurowitz, F. Über Globin und seine hämaffine Gruppe. Ztschr. f. physiol. Chem. *232*: 146. 1935. 28, 49, 50

232. Heidelberger, M., and W. W. Palmer. The preparation and properties of thyroglobulin. J. Biol. Chem. *101*: 433. 1933. 33, 34

233. Heidelberger, M., and K. O. Pedersen. The molecular weight and isoelectric point of thyroglobulin. J. Gen. Physiol. *19*: 95. 1935. 37

234. Heidelberger, M., and T. Svedberg. The molecular weight of thyroglobulin. Science *80*: 414. 1934. 35

235. Hektoen, L., A. J. Carlson, and K. Schulhof. Further attempts to increase experimentally the hormone output by the thyroid gland. Am. J. Physiol. *81*: 661. 1927. 70, 166

236. Hektoen, L., P. H. Kanai, and L. R. Dragstedt. A study of protein absorption from the digestive tract by the precipitin test. J.A.M.A. *84*: 114. 1925. 82

237. Henze, M. Zur Chemie des Gorgonins und der Jodgorgosäure. Ztschr. f. physiol. Chem. *38*: 60. 1903. 31

238. ——Zur Kenntnis der jodbindenden Gruppe der natürlich vorkommenden Jodeiweisskörper. Die Konstitution der Jodgorgosäure. Ztschr. f. physiol. Chem. *51*: 64. 1907. 53

239. Hertz, S., and E. G. Oastler. Assay of blood and urine for thyreotropic hormone in thyrotoxicosis and myxedema. Endocrinology *20*: 520. 1936. 94, 133, 286

240. Hertz, S., A. Roberts, and R. D. Evans. Radioactive iodine as an indicator in the study of thyroid physiology. Proc. Soc. Exper. Biol. & Med. *38*: 510. 1938. 213, 218, 266

241. Hertz, S., A. Roberts, J. H. Means, and R. D. Evans. Radioactive iodine as an indicator in thyroid physiology. Iodine collection by normal and hyperplastic thyroids in rabbits. Am. J. Physiol. *128*: 565. 1940. 7, 24, 100, 107, 212, 216, 219, 221, 222, 224, 230, 233, 256

242. Herxheimer, H., E. Mislowitzer, and L. Stanoyévitch. Sur la quantité d'iode présente dans le sang pendant le travail musculaire intensif. Compt. rend. Soc. de biol. *115*: 886. 1934. 80

243. Hevesy, G. V. Radiochemical methods in chemistry, physics, and biology. Ztschr. f. Elektrochem. u. eng. physik. Chem. *38*: 504. 1932. 213

244. Hicks, C. S. Tautomerism in the thyroxin molecule. J. Chem. Soc. London. p. 643. 1926. 115

245. Hirsch, O. Beitrag zum Basedowproblem. Deutsches Arch. f. klin. Med. *168*: 331. 1930. 67

246. —— Über Beziehungen zwischen Eierstock und Schilddrüse bei der Basedowschen Krankheit. Deutsches Arch. f. klin. Med. *171*: 44. 1931. 21

247. Hofmeister, F. Zur Frage nach den Folgezuständen der Schilddrüsenexstirpation. Deutsche med. Wchnschr. *22*: 354. 1896. 29

248. —— Untersuchungen über die Proteinstoffe. I. Ueber jodirtes Eieralbumin. Ztschr. f. physiol. Chem. *24*: 159. 1898. 27, 28, 45, 53

249. Hofrichter, B. Über den Nutzen der Schilddrüse. Deutsches Arch. f. Physiol. *6*: 161. 1820. 146

250. Höjer, J. A. Bemerkungen zu der Bestimmung kleiner Jodmengen nach Dupré-Winklers Methode. Biochem. Ztschr. *205*: 273. 1929. 124

251. Holst, J., and G. Lunde. Intermediate iodine metabolism during the preoperative treatment of exophthalmic goiter. Am. J. Surg. *7*: 39. 1929. 89, 121

252. Holst, J., G. Lunde, K. Closs, and O. Chr. Pedersen. Über den inneren Jodstoffwechsel bei primären Thyreotoxikosen (Primär-Basedow). Klin. Wchnschr. *7*: 2287. 1928. 89

253. Hoskins, M. M. The effect of acetyl thyroxine on the newborn white rat. J. Exper. Zoöl. *48*: 373. 1927. 112

254. Hoskins, R. G. Rothschild Memorial Lecture in connection with the New York Academy of Medicine Symposium on Endocrinology. New York. 1939. 252

255. —— Thyroid secretion as a factor in adrenal activity. J.A.M.A. *55*: 1724. 1910. *See also* Congenital thyroidism: An experimental study of the thyroid in relation to other organs of internal secretion. Am. J. Physiol. *26*: 426. 1910. 142

256. Houssay, B. A., P. Mazzocco, and A. Biasotti. Hypophyse et thyroïde. L'iode sanguin des chiens hypophysoprives. Compt. rend. Soc. de biol. *108*: 915. 1931. 118, 126, 176

257. Hudson, G. E. The permeability of the placenta to inorganic iodides. J.A.M.A. *97*: 1513. 1931. 13

258. Hundeshagen, F. Über jodhaltige Spongien und Jodspongin. Ztschr. f. ang. Chem. p. 473. 1895. 27, 31

259. Hutchison, R. The chemistry of the thyroid gland and the nature of its active constituent. J. Physiol. *20*: 474. 1896. 4, 29, 32

260. —— Further observations on the chemistry and action of the thyroid gland. J. Physiol. *23*: 178. 1898–99. 27, 32

261. Isenbruch, J. H. Über den Jodgehalt menschlicher Organe. Schrift. d. Naturf. Gesellsch. in Danzig *19*: 1. 1929–30. 193

262. Jackson, C. M., and M. T. P'An. The effects of dietary deficiency of iodin upon the thyroid and parathyroid glands in the rat. Endocrinology *16*: 146. 1932. 182

263. Jahn, D., and O. Kesselkaul. Die Abhängigkeit des Blutjodspiegels von der Tätigkeit der Ovarien. Deutsches Arch. f. klin. Med. *161*: 143. 1928. 151, 152

264. Joll, C. A. Diseases of the Thyroid Gland with Special Reference to Thyrotoxicosis. St. Louis, C. V. Mosby Company. 1939. (See especially data of W. M. Ash, p. 257.) 254

265. Jones, M. S. A study of thyrotropic hormone in clinical states. Endocrinology *24*: 665. 1939. 260

266. Junkmann, K., and W. Schoeller. Über das thyreotrope Hormon des Hypophysenvorderlappens. Klin. Wchnschr. *11*: 1176. 1932. 133, 287

267. Justus, J. Ueber den physiologischen Jodgehalt der Zelle. Virchows Arch. f. path. Anat. *170*: 501. 1902. *See also*, ibid., *176*: 1. 1904. 6

268. Karp, L., and B. Kostkiewicz. Goître colloïdal expérimental provoqué par la folliculine. Compt. rend. Soc. de biol. *114*: 1339. 1933. 156

269. Kassell, B., and E. Brand. The photometric determination of cystine, cysteine, ascorbic acid, and related compounds with phosphotungstic acid. J. Biol. Chem. *125*: 115. 1938. 38, 284

270. —— The determination of methionine, cysteine, and sulfate in proteins after hydrolysis with hydriodic acid. J. Biol. Chem. *125*: 145. 1938. 38

271. Kendall, E. C. The isolation in crystalline form of the compound containing iodin, which occurs in the thyroid. Its chemical nature and physiologic activity. J.A.M.A. *64*: 2042. 1915. 4

272. —— Isolation of the iodine compound which occurs in the thyroid. J. Biol. Chem. *39*: 125. 1919. 51

273. —— The physiologic action of thyroxin. Endocrinology *3*: 156. 1919. 4, 205

274. —— Thyroxine. New York, Chemical Catalog Company. 1921 (revised in 1929). 110, 112, 114

275. —— The consideration of some of the glands of internal secretion from a chemical viewpoint. Endocrinology *15*: 357. 1931. 112

276. Kendall, E. C. Abstract of discussion on papers of Collip et al. J.A.M.A. *104*: 978. 1935.	118
277. Kendall, E. C., and A. E. Osterberg. The chemical identification of thyroxin. J. Biol. Chem. *40*: 265. 1919. *See also* E. C. Kendall, and D. G. Simonsen. Seasonal variations in the iodine and thyroxine content of the thyroid gland. Ibid. *80*: 357. 1928.	47, 52, 102, 183
278. Kendall, E. C., and F. S. Richardson. Determination of iodine in blood and in animal tissues. J. Biol. Chem. *43*: 161. 1920.	71
279. Kimball, O. P. Prevention of goiter in Michigan and Ohio. J.A.M.A. *108*: 860. 1937.	192
280. —— Twenty years in the prevention of goiter (1916–1936). Tr. Third Internat. Goiter Conf. and Am. A. Study Goiter. pp. 57–63. 1938.	147, 254
281. King, E. L., and J. S. Herring. Hypothyroidism in the causation of abortion, especially of the "missed" variety. J.A.M.A. *113*: 1300. 1939.	157
282. Kisch, F. Über das Verhalten des Blutjodspiegels Kreïslaufkranker. Klin. Wchnschr. *13*: 1317. 1934.	244
283. —— Zur Klinik atypischer Blutjodwerte. Klin. Wchnschr. *15*: 342. 1936.	96
284. Klein, W., A. Pfeiffer, and G. Hermann. Der chemische und histologische Nachweis der Jodspeicherung in der Schilddrüse. Biochem. Ztschr. *225*: 344. 1930.	100
285. Klose, H., and G. Büttner. Kachexia strumipriva in Handbuch d. inn. Sekretion *3*: 591. 1927. Leipzig, M. Hirsch, C. Kabitzsch.	118
286. Knop, cited by F. Blum and E. Strauss. Centr. 1875: 395, 411, and 420. Cf. Ztschr. f. physiol. Chem. *112*: 111. 1920–21.	27
287. Kocher, A. Die histologischen und chemische Veränderungen der Schilddrüse bei Morbus Basedowii und ihre Beziehung zur Funktion der Drüse. Virchows Arch. f. path. Anat. *208*: 86. 1912. *See also* XIX. Ueber die Ausscheidung des Jods im menschlichen Harn und ihre Beziehung zum Jodgehalt und zur Verkleinerung der Strumen. Ein Beitrag zur Physiologie der Schilddrüse. Mitt. a. d. Grenzgeb. d. Med. u. Chir. *14*: 359. 1905.	119, 155
288. —— Resultate chemischer und histologischer Untersuchungen über die Art und die Bedeutung des Jods in der Schilddrüse und in der Struma. Ber. ü. d. internat. Krophkonf., Bern. pp. 183–189. 1927.	68, 107
289. Kocher, Th. Ueber Basedow. Arch. f. klin. Chir. *96*: 403. 1911.	122
290. Koelsche, G. A., and E. C. Kendall. The relation of the suprarenal cortical hormone to nitrogen metabolism in experimental hyperthyroidism. Am. J. Physiol. *113*: 335. 1935.	142
291. Kögl, F., and H. Erxleben. Zur Ätiologie der malignen Tumoren. I. Mitteilung. Über die Chemie der Tumoren. Ztschr. f. physiol. Chem. *258*: 57. 1939.	13

292. Kommerell, B. Über den Nachweis von Schilddrüsenhormon in der Muskulatur. Arch. f. exper. Path. u. Pharmakol. *161*: 141. 1931. 18

293. —— Schilddrüse und Arbeitsstoffwechsel. Pflüger's Arch. f. d. ges. Physiol. *227*: 1. 1931. 194

294. —— Zur Frage der Wirkung des Dijodtyrosins auf den thyreotoxischen Stoffwechsel. Arch. f. exper. Path. u. Pharmakol. *165*: 169. 1932. 122

295. Koppenhöfer, G. F. Untersuchungen über den Jodgehalt der menschlichen Hypophyse. Ztschr. f. d. ges. exper. Med. *94*: 57. 1934. 19

296. Kraft, K., and R. May. Beiträge zur Biochemie des Fluors. II. Fluorbestimmungen an Blut und Wässern. Ztschr. f. physiol. Chem. *246*: 233. 1937. 112, 192

297. Kraft, R. Ueber den Einfluss der Schilddrüse auf die Verteilung von Jod, Glukose und Aminosaüren im Blute. Mitt. a. d. Grenzgeb. d. Med. u. Chir. *40*: 433. 1927–28. 23, 84

298. Krayer, O. Über Verteilung und Ausscheidung des Jodes nach Zufuhr von Schilddrüsenstoffen. Arch. f. exper. Path. u. Pharmakol. *128*: 116. 1928. 184, 203, 204, 205

299. —— Ist die Integrität der sympathischen Schilddrüseninnervation notwendig für die thyreotrope Wirkung des Hypophysenvorderlappens? Arch. f. exper. Path. u. Pharmakol. *171*: 473. 1933. 166

300. Krogh, A. The use of isotopes as indicators in biological research. Science *85*: 187. 1937. 213

301. Krogh, M., and A.-L. Lindberg. Studies on the thyroid gland. II. The physiological activity of iodine in thyroxin and in normal and pathological thyroid glands. Acta path. et microbiol. Scandinav. *9*: 21. 1932. 89, 119, 130

302. Krogh, M., and H. Okkels. L'hormone thyréostimulante préhypophysaire est-elle présente dans l'urine? Compt. rend. Soc. de biol. *113*: 635. 1933. 125

303. Kunde, M. M., F. E. D'Amour, A. J. Carlson, and R. G. Gustavson. Studies on metabolism. VIII. The effect of estrin injections on the basal metabolism, uterine endometrium, lactation, mating and maternal instincts in the adult dog. Am. J. Physiol. *95*: 630. 1930. 159

304. Kurajeff, D. Ueber Einführung von Jod in das krystallisirte Serum- und Eieralbumin. Ztschr. f. physiol. Chem. *26*: 462. 1899. 46

305. —— Ueber das Jodprodukt des Oxyhämoglobins. Ztschr. f. physiol. Chem. *31*: 527. 1901. 50

306. Kuschinsky, G. Über die Bedingungen der Sekretion des thyreotropen Hormons der Hypophyse. Arch. f. exper. Path. u. Pharmakol. *170*: 510. 1933. 137

307. Laquer, F. Hormone und innere Sekretion. Dresden and Leipzig, Verlag Theodor Steinkopff. 1934. 113

308. Lawrence, E. O. Artificial radioactivity. Ohio J. Sc. *35*: 388. 1935. 213

309. Leipert, T. Die Bestimmung kleinster Jodmengen in organischem Material. Biochem. Ztschr. *261*: 436. 1933. 271, 281

310. —— Zur Kenntnis des physiologischen Blutjodspiegels. Biochem. Ztschr. *270*: 448. 1934. 70, 151, 153

311. —— Zur Kenntnis des Blutjods. Biochem. Ztschr. *293*: 99. 1937. 74

312. —— Das Jod. Mikrochim. Act. *3*: 73. 1938. 4, 218, 276

313. Leitch, I. Variations in blood iodine associated with changes in ovarian activity. I. Intern. Kropfkorf. Bern. 1927. 153

314. Leitch, I., and J. McA. Henderson. The estimation of iodine in foodstuffs and body fluids. Biochem. J. *20*: 1003. 1926. *See also* W. Anderson, and I. Leitch. Note on the effect of anaesthetics on blood iodine. Lancet *2*: 1391. 1927. 153, 189, 205

315. Leland, J. P., and G. L. Foster. A method for the determination of thyroxine in the thyroid. J. Biol. Chem. *95*: 165. 1932. 20, 24, 75, 101, 284–286

316. Lerman, J. Personal communication. 1939. *See also* Iodine components of the blood. Circulating thyroglobulin in normal persons and in persons with thyroid disease. J. Clin. Investigation *19*: 555. 1940. 75

317. Lerman, J., and J. H. Means. Iodin in exophthalmic goiter. A comparison of the effect of ethyl iodid and potassium iodid with that of Lugol's solution. Am. J. M. Sc. *181*: 745. 1931. 100

318. Lerman, J., and W. T. Salter. The calorigenic action of thyroid and some of its active constituents. Endocrinology *18*: 317. 1934. 76, 109, 116, 185

319. —— The calorigenic activity of thyroid iodine at different levels of metabolism. J. Clin. Investigation (Proc.) *16*: 678. 1937. 108, 109

320. —— Iodinated protein in human athyreosis. II. The production of physiological activity by simple iodination of serum protein. J. Clin. Investigation (Proc.) *18*: 493. 1939. 60, 110

321. —— The relief of myxedema with proteins of extrathyroidal origin. Endocrinology *25*: 712. 1939. 18, 57, 59, 76, 111

322. Levine, H., R. E. Remington, and H. von Kolnitz. (*a*) Studies on the relation of diet to goiter. I. A dietary technic for the study of goiter in the rat. (*b*) Studies on the relation of diet to goiter. II. The iodine requirement of the rat. J. Nutrition *6*: 325–347. 1933. 182

323. Lewenfisz, H. Rentgenoterapja nadnerczy, jako jedna z metod leczniczych choroby Basedowa. Polska gaz. lek. *13*: 944. 1934. 143

324. Lewis, A., N. Samuel, and J. Galloway. A study of cretinism in London: with especial reference to mental development and problems of growth. Lancet *1*: 1505. 1937. 144

325. Liebrecht, A. Ueber Jodderivate von Eiweisskörpern (Caseïn). Ber. d. deutsch. chem. Gesellsch. *30*: 1824. 1897. 52, 55

326. Lipschitz, W. Die Bedeutung des "inneren Kreislaufes" für Krystalloide, speziell das Jodion. Klin. Wchnschr. *8*: 116. 1929. 23, 81, 203

327. Litchfield, H. R. The value of blood iodine determination from a clinical viewpoint. Tr. Third Internat. Goiter Conf. and Am. A. Study Goiter. pp. 414–423. 1938. *See also* M. Rec. *148*: 365. 1938. 96

328. Litzka, G. Die antithyreotische Wirkung des Fluortyrosins. Arch. f. exper. Path. u. Pharmakol. *183*: 436. 1936. 112

329. Livingood, J. J., and G. T. Seaborg. Radioactive isotopes of iodine. Physical Rev. *54*: 775. 1938. 213, 215, 216

330. Loeb, L. The specificity in the action of the anterior pituitary of different mammals as well as of urine of pregnant women on the sex organs and thyroid glands of immature female guinea pigs. Endocrinology *16*: 129. 1932. 132

331. —— Schilddrüse, Jod und Hypophysenvorderlappen. Klin. Wchnschr. *11*: 2121. 1932. 106, 107

332. —— The thyroid stimulating hormone of the anterior pituitary gland. Ann. Int. Med. *9*: 13. 1935. 122, 125, 128, 143

333. Loeb, L., R. B. Bassett, and H. Friedman. Further investigations concerning the stimulating effect of anterior pituitary gland preparation on the thyroid gland. Proc. Soc. Exper. Biol. & Med. *28*: 209. 1930. 132

334. Loeb, O. Die Jodverteilung nach Einfuhr verschiedener Jodverbindungen. Arch. f. exper. Path. u. Pharmakol. *56*: 320. 1907. 23

335. Loeser, A. Beziehungen zwischen der thyreotropen Substanz des Hypophysenvorderlappens und den Nebennieren. Klin. Wchnschr. *12*: 1614. 1933. 143

336. —— Die Umstimmung der Schilddrüsentätigkeit durch Jod. Klin. Wchnschr. *13*: 533. 1934. 105, 107, 133

337. —— Die schilddrüsenwirksame Substanz des Hypophysenvorderlappens. I. Arch. f. exper. Path. u. Pharmakol. *176*: 697. 1935. II. Wechselbeziehungen zwischen der schilddrüsenwirksamen Substanz des Hypophysenvorderlappens und anderen innersekretorischen Organen. Arch. f. exper. Path. u. Pharmakol. *176*: 729. 1934. 152, 154, 155

338. —— Hyperthyreose und thyreotropes Hormon der Hypophyse. Klin. Wchnschr. *16*: 913. 1937. 85, 125, 127, 129

339. Loeser, A., and K. W. Thompson. Hypophysenvorderlappen, Jod und Schilddrüse. Der Mechanismus der Schilddrüsenwirkung des Jods. Endokrinologie *14*: 144. 1934. *See also* The anterior pituitary, iodine, and the thyroid gland. Internat. Clin. *45*, *III*: 149. 1935. 122, 128, 132, 137

340. Loewi, O. Über humorale Übertragbarkeit der Herznervenwirkung. Pflüger's Arch. f. d. ges. Physiol. *193*: 201. 1922. 171

341. Logan, M. A., J. E. VanderLaan, and W. P. VanderLaan. The effect of the thyrotropic hormone on the composition and metabolism of the thyroid gland. Proc. Am. Soc. Biol. Chem. XXIV, J. Biol. Chem. *34*: lxii. 1940. 11, 127

342. **Löhr, H.** Beiträge zur Kenntnis des Jodstoffwechsels. I. Mitt.: Blutjodstudien unter physiologischen Verhältnissen. Arch. f. exper. Path. u. Pharmakol. *180*: 332. 1936. II. Mitt.: Die Beeinflussung des Blutjodspiegels durch Ascorbinsäure. Ibid. p. 344. 72, 190

343. —— Über den Jodgehalt des Zwischenhirns und seine Beziehung zur Schilddrüse. Klin. Wchnschr. *16*: 1661. 1937. *See also* H. Löhr, and H. Willmanns. Über die Verteilung des Jods im Gehirn unter normalen Bedingungen und nach Jodzufuhr. Verhandl. d. deutsch. Gesellsch. f. inn. Med., Kong. Diskussionsvortrag *49*: 293. 1937. 19, 20, 177

344. **Ludwig, W., and P. v. Mutzenbecher.** Über die Entstehung von Thyroxin durch Jodierung von Eiweiss. Ztschr. f. physiol. Chem. *244*: IV. 1936. *See also* Die Darstellung von Thyroxin, Monojodtyrosin und Dijodtyrosin aus jodiertem Eiweiss. Ztschr. f. physiol. Chem. *258*: 195. 1939. 7, 18, 29, 46, 51, 57, 110

345. **Lunde, G.** Untersuchungen über den Jodstoffwechsel. I. Über die Jodausschiedung durch den Harn von Bewohnern eines norwegischen Kropfgebietes. Biochem. Ztschr. *193*: 94. 1928. 182

346. **Lunde, G., K. Closs, and O. Chr. Pedersen.** Untersuchungen über den Jodstoffwechsel. III. Untersuchungen über den Blutjodspiegel bei der primären Thyreotoxikose. Biochem. Ztschr. *206*: 261. 1929. 76, 82, 97

347. **Lunde, G., K. Closs, and K. Wülfert.** Untersuchungen über den Jodstoffwechsel. II. Über den Jodgehalt von normalen und pathologisch veränderten Schilddrüsen. Biochem. Ztschr. *206*: 248. 1929. 16, 73, 119

348. **Lunde, G., and K. Wülfert.** Untersuchungen über die Jodhaltigen Körper der Thyreoidea. I. Endokrinologie *4*: 321. 1929. 113

349. —— Untersuchungen über den Jodstoffwechsel. IV. Über die Verteilung des Jods in normalen und jodbehandelten hyperthyreotischen Schilddrüsen. Endokrinologie *7*: 327. 1930. 120

350. **Magnus–Levy, A.** Über Myxödema. Ztschr. f. klin. Med. *52*: 201. 1904. 120

351. **Magoun, H. W., C. Fisher, and S. W. Ranson.** The neurohypophysis and water exchange in the monkey. Endocrinology *25*: 161. 1939. 172

352. **Marine, D.** Quantitative studies on the *in vivo* absorption of iodine by dogs' thyroid glands. J. Biol. Chem. *22*: 547. 1915. *See also* Etiology and prevention of simple goiter. Medicine *3*: 453. 1924. 23, 100, 253

353. —— Control of compensatory hyperplasia of the thyroid of guinea-pigs by the administration of iodine. Arch. Path. *2*: 829. 1926. 232

354. —— Studies on the etiology of goiter including Graves' disease. Ann. Int. Med. *4*: 423. 1930. 143, 190

355. —— I. The physiology and principal interrelations of thyroid. J.A.M.A. *104*: 2250. 1935. II. The pathogenesis and prevention of simple or endemic goiter. Ibid. p. 2334. *See also* Physiology and principal interrelations of the thyroid gland. Bull. New York Acad. Med. *15*: 790. 1939. 137, 147, 188, 224, 256

356. ——Does the study of experimental exophthalmos offer any clue to the fundamental nature of Graves' disease? Tr. Third Internat. Goiter Conf. and Am. A. Study Goiter. pp. 7–16. 1938. *See also* Studies on the pathological physiology of the exophthalmos of Graves' disease. Ann. Int. Med. *12*: 443. 1938. 260

357. Marine, D., A. Cipra, and L. Hunt. Influence of the thyroid gland on the increased heat production occurring during pregnancy and lactation. J. Metab. Research *5*: 277. 1924. 155

358. Marine, D., and O. P. Kimball. The prevention of simple goiter in man. J. Lab. & Clin. Med. *3*: 40. 1917. 253

359. Marine, D., and C. H. Lenhart. Further observations on the relation of iodin to the structure of the thyroid gland in the sheep, dog, hog, and ox. Arch. Int. Med. *3*: 66. 1909. 186

360. ——Effects of the administration or the withholding of iodin-containing compounds in normal, colloid, or actively hyperplastic (parenchymatous) thyroids of dogs. Arch. Int. Med. *4*: 253. 1909. 12, 13, 106

361. Marine, D., and J. M. Rogoff. How rapidly does the intact thyroid gland elaborate its specific iodin-containing hormone? J. Pharmacol. & Exper. Therap. *9*: 1. 1916. 24

362. Marine, D., and S. H. Rosen. The exophthalmos of Graves' disease: its experimental production and significance. Am. J. M. Sc. *188*: 565. 1934. 190, 223

363. ——The effect of the thyrotropic hormone on auto- and homeotransplants of the thyroid, and its bearing on the question of secretory nerves. Am. J. Physiol. *107*: 677. 1934. 175

364. Marine, D., and W. W. Williams. The relation of iodin to the structure of the thyroid gland. Arch. Int. Med. *1*: 349. 1908. 123

365. Martin, N. H. Note on iodine content of thyroid gland. Pharm. J. *89*: 144. 1912. 72

366. Maruno, Y. Experimental studies on the halogen-excretion from the liver. IV. The influence of cholagoga and functional disturbance of the liver upon the iodine excretion from the liver. Jap. J. Gastroenterol. *3*: 111. 1931. Cited in Ber. ü. d. ges. Physiol. *63*: 457. 1932. 204

367. Mârza, V. D., and A. V. Blinov. L'hypophyse antérieure et la thyroïde au cours de l'ovulation. Compt. rend. Soc. de biol. *121*: 1438. 1936. 157

368. Mason, J. B., J. Markowitz, and F. C. Mann. A plethysmographic study of the thyroid gland of the dog. Am. J. Physiol. *94*: 125. 1930. 167

369. Mathews, N. L., G. M. Curtis, and W. R. Brode. Determination of iodine in biological materials. Refinements of the chromium trioxide oxidation method. Indust. & Engin. Chem., Analytical Ed. *10*: 612. 1938. 218, 271, 276

370. Man, E. B. Personal communication. *See also* D. S. Riggs, and E. B. Man. A permanganate acid ashing micromethod for iodine determinations. I. Values in blood of normal subjects. J. Biol. Chem. *134*: 193. 1940. 276

371. Maurer, E. Gibt es einer unter physiologischen Verhältnissen erfolgende Tätigkeit der fetalen Schilddrüse? Ztschr. f. Kinderh. *63*: 163. 1927. 13

372. Maurer, E., and St. Diez. Untersuchungen über das Vorkommen von Jod im menschlichen und tierischen Organismus. München. med. Wchnschr. *73*: 17. 1926. 151

373. —— Zur Kenntnis des Jods als biogenes Element. IV. Die biochemische Bedeutung des Jodgehalts in Früh- und Dauermilch. Biochem. Ztschr. *178*: 161. 1926. 70

374. Maurer, E., and H. Ducrue. Zur Kenntnis des Jods als biogenes Element. XII. Der Jodgehalt im normalen tierischen Organismus. Biochem. Ztschr. *193*: 356. 1928. XIII. Die Beeinflussbarkeit des Jodgehaltes im tierischen Organismus durch perorale Zufuhr geringer Mengen anorganisch gebundenen Jods. Ibid. p. 360. XIV. Die Beeinflussung des Jodblutspiegels durch einmalige Zufuhr anorganisch gebundenen Jods. Ibid. p. 364. 21, 22, 23, 73, 84, 100, 105, 148, 204

375. Maurer, E., H. Ducrue, and W. Palasoff. Untersuchungen über das Vorkommen von Jod im menschlichen und tierischen Organismus. II. München. med. Wchnschr. *74*: 271. 1927. 21, 149

376. McCarrison, R. Recherches récentes sur l'étiologie du goitre. 2-e. Conf. Intern. du goître, Bern. Presse méd. *41*: 1612. 1933. 147, 254

377. McClendon, J. F. Iodine and the Incidence of Goiter. Minneapolis, Univ. of Minnesota Press. 1939. *See also* Iodine and goiter with especial reference to the far east. J. Biol. Chem. *102*: 91. 1933. 4, 16, 73, 77, 146, 188, 189, 270, 271, 283

378. McClendon, J. F., R. E. Remington, H. v. Kolnitz, and R. Rufe. The determination of traces of iodine. III. Iodine in milk, butter, oil, and urine. J. Am. Chem. Soc. *52*: 541. 1930. 71, 193, 194

379. McClendon, J. F., and R. White. Iodine in samples containing little organic matter other than urea. Proc. Soc. Exper. Biol. & Med. *39*: 215. 1938. 271

380. McCullagh, D. R. A new method for the determination of iodine. J. Biol. Chem. *107*: 35. 1934. 71

381. —— Studies in blood iodine by the use of a new chemical method. Cleveland Clin. Quart. *2* (No. 1): 15. 1935. 67, 84, 85, 194

382. McCullagh, E. P., and D. R. McCullagh. Clinical experiences in the use of determinations of blood iodine. Arch. Int. Med. *57*: 1061. 1936. 80, 83

383. Means, J. H. The diagnostic use of iodine in thyrotoxicosis. Ann. Int. Med. *7*: 439. 1933. 120, 253

384. —— The Thyroid and Its Diseases. Philadelphia, J. B. Lippincott. 1937. 108, 116, 192, 237, 240, 251, 253

385. —— On the pathogenesis of Graves' disease. Proc. Staff Meet., Mayo Clin. *12*: 11. 1937. 238

386. —— Report on medical progress. Diseases of the thyroid gland. New England J. Med. *221*: 820. 1939. 86, 118, 121

387. —— Personal communication. 1939. 120

388. Means, J. H., and J. Lerman. The action of iodine in thyrotoxicosis. J.A.M.A. *104*: 969. 1935. — 120, 121, 122, 256

389. —— The curves of thyroxine decay in myxedema and of iodine response in thyrotoxicosis: their similarity and its possible significance. Ann. Int. Med. *12*: 811. 1938. — 108, 109

390. Means, J. H., J. Lerman, and W. T. Salter. The rôle of thyroxin iodine and total organic iodine in the calorigenic action of whole thyroid gland. J. Clin. Investigation *12*: 683. 1933. — 116, 186

391. Means, J. H., W. O. Thompson, and P. K. Thompson. On the nature of the iodine reaction in exophthalmic goiter: with particular reference to the effect of iodine late in the course of the disease. Tr. A. Am. Physicians *43*: 146. 1928. — 255

392. Membrives, J. R. Thyroïde et pouvoir gonadotropique de l'hypophyse du rat. Compt. rend. Soc. de biol. *127*: 695. 1938. — 158

393. Meyer, A. E. Personal communication. 1939. *See also* A. E. Meyer, and H. Danow. Variability of action on heart rate compared with metabolic effect of various thyroid preparations.. Proc. Soc. Exper. Biol. & Med. *44*: 439. 1940. — 114, 116

394. Meyer, A. E., and M. Yost. The stimulating action on metabolism and heart beat of various thyroid preparations, determined in the thyroidectomized rat. Endocrinology *24*: 806. 1939. — 114, 116

395. Meyer, H. E. Ein Beitrag zur Frage der Jodverhältnisse in der Schilddrüse und in deren Extrakten mit besonderer Berücksichtigung des anorganischen, Lipoid- und Eiweissjodes. Ztschr. f. physiol. Chem. *156*: 231. 1926. — 24

396. Modell, W. Observations on the structure of the blood vessels within the thyroid gland of the dog. Anat. Rec. *55*: 251. 1933. — 166

397. Moebius, P. J. Schilddrüsentheorie. Schmidt's Jahrb. *210*: 237. 1886. Cited by the author in Die Basedow'sche Krankheit. Wien. 1896. In Nothnagel's Specielle Pathologie und Therapie, vol. 22. — 89, 112

398. Moebius, W., and F. A. Nolte. Verhalten von Grundumsatz und Blutjod bei Thyreotoxicosen. Ztschr. f. klin. Med. *128*: 174. 1935. — 86

399. Moore, C. R., and D. Price. The question of sex hormone antagonism. Proc. Soc. Exper. Biol. & Med. *28*: 38. 1930. — 158

400. Müller, C., and Th. v. Fellenberg. Untersuchungen über das Schicksal des Thyroxins im Organismus unter experimentellen Bedingungen. Mitt. a. d. Grenzgeb. d. Med. u. Chir. *42*: 661. 1932. — 23

401. Mutzenbecher, P. von. Über die Bildung von Thyroxin aus Dijodtyrosin. Ztschr. f. physiol. Chem. *261*: 253. 1939. — 110

402. Naffziger, H. C. Progressive exophthalmos following thyroidectomy: its pathology and treatment. Tr. Am. S. A. *49*: 168. 1931. — 260

403. —— Pathologic changes in the orbit in progressive exophthalmos; with special reference to alterations in the extra-ocular muscles and the optic disks. Arch. Ophth. *9*: 1. 1933. — 260

404. NAKAMURA, U. Experimental study of the thyroid function during pregnancy, parturition, and puerperium. Jap. J. Obst. & Gynec. *15*: 114, 190. 1932. 154, 156

405. —— Experimental study of the thyroid function during pregnancy, parturition, and puerperium. Jap. J. Obst. & Gynec. *16*: 244, 246, 470. 1933. 154, 156

406. NATHANSON, I. T., AND R. RAWSON. Personal communication. 1939. 128, 156

407. NEUBERGER, A. Electrometric titration of zein and iodozein. Biochem. J. *28*: 1982. 1934. 28, 47

408. NEWTON, A. Toxic goitre, with special reference to end-results. M. J. Australia *2*: 265. 1938. 261

409. —— The hospital management of patients suffering from thyrotoxicosis. Roy. Melbourne Hosp. Clin. Rep. *9*: 133. 1938. 265

410. NIÈPCE, B. Traité du goitre et du crétinisme. Paris. 1851. 125

411. NOETHER, P. Jod im Kolloid der Rinderhypophyse. Klin. Wchnschr. *11*: 462. 1932. 19, 138

412. NONIDEZ, J. F. Innervation of the thyroid gland. III. Distribution and termination of the nerve fibers in the dog. Am. J. Anat. *57*: 135. 1935. 165, 166

413. —— The nervous "terminal reticulum." A critique. II. Observations on the thyroid and the liver. Anat. Anz. *84*: 1. 1937. 166

414. OEHME, C. Zur antithyreoiden Wirkung der Nebennierenrinde. Klin. Wchnschr. *15*: 512. 1936. 142

415. OLESEN, R., AND N. E. TAYLOR. Incidence of endemic thyroid enlargement in Connecticut. U. S. Pub. Health Rep. *41*: 1695. 1926. 146

416. ORD, W. M. On myxoedema, a term proposed to be applied to an essential condition in the "cretinoid" affection occasionally observed in middle-aged women. Tr. Med.-Chir. Soc. London *61*: 57. 1878. 84

417. ORD, W. M., AND E. WHITE. On certain changes observed in the urine in myxoedema after the administration of glycerine extract of thyroid gland. Brit. M. J. *ii*: 217. 1893. 185

418. ORR, J. B., AND I. LEITCH. Iodine in nutrition. A review of existing information. Med. Research Council Special Report, Series 123. London. 1929. 21, 186

419. OSBORNE, E. D. Iodin in the cerebrospinal fluid with special reference to iodid therapy. J.A.M.A. *76*: 1384. 1921. *See also* Pharmacology and therapeutics of iodids. Ibid. *79*: 615. 1922. 67, 201

420. OSWALD, A. Über das Verhalten von 3,5-Dijod-l-tyrosin und 3,5-Dijod-r-tyrosin im tierischen Organismus. Ztschr. f. physiol. Chem. *62*: 399. 1909. 203

421. —— Gewinnung von 3,5-Diiodotyrosin aus Jodeiweiss. Ztschr. f. physiol. Chem. *70*: 310. 1911. II. Gewinnung desselben aus Jodglidin. Ibid. *71*: 200. 1911. III. Gewinnung desselben aus Jodcasein. Ibid. *74*: 290. 1911. IV. Die Verhältnisse beim Gorgonin und Spongin. Ibid. *75*: 353. 1911. 7, 27, 51

422. —— Über die Wirkung der Schilddrüse auf den Blutkreislauf. Pflüger's Arch. f. d. ges. Physiol. *164*: 506. 1916. 161

423. Paal, H. Über die hormonale Beeinflussung des Stoffwechsels von überlebendem Schilddrüsengewebe. Arch. f. exper. Path. u. Pharmakol. *173*: 513. 1933. 129

424. —— Die Bedeutung der Schilddrüse für die Atmung von Warmblüter-Gewebsschnitten. Klin. Wchnschr. *13*: 207. 1934. 111

425. Palmer, W. W., and J. P. Leland. Comparative calorigenic action of normal and pathological thyroid glands administered in equithyroxine doses. J. Clin. Investigation *14*: 619. 1935. *See also* W. W. Palmer, J. P. Leland, and A. B. Gutman. The microdetermination of thyroxine in the thyroid gland of the new-born. J. Biol. Chem. *125*: 615. 1938. 75, 119, 286

426. Parhon, C. I., and L. Ballif. Syndrome basedowien évoluant vers le myxoedème à la suite du traitement par la diiodotyrosine. Bull. et mém. Soc. méd. d. hôp. de Paris *48*: 601. 1932. 120

427. Pauly, H. Über jodierte Abkömmlinge des Imidazols und des Histidins. Ber. d. deutsch. chem. Gesellsch. *43*: 2243. 1910. 28, 54

428. Perkin, H. J. Personal communication. 1939. 73, 74

429. Perkin, H. J., and B. R. Brown. The influence of the thyroid gland and of the ovary on the metabolism of iodine. Endocrinology *22*: 538. 1938. 21, 149, 255

430. Perkin, H. J., B. R. Brown, and J. Lang. The blood iodine content of normal and thyrotoxic individuals: an iodine tolerance test. Canad. M. A. J. *31*: 365. 1934. 270

431. Perkin, H. J., and L. M. Hurxthal. The blood iodine level, before and after subtotal thyroidectomy for hyperthyroidism. New England J. Med. *215*: 698. 1936. *See also* The fractionation of the iodine of the blood in thyroid disease. J. Clin. Investigation *18*: 733. 1939. 30, 210, 251

432. Perkin, H. J., F. H. Lahey, and R. B. Cattell. Blood iodine studies in relation to thyroid disease. Basic concept of the relation of iodine to the thyroid gland; an iodine tolerance test. New England J. Med. *214*: 45. 1936. 79, 85, 104, 266

433. Perkin, H. J., and F. H. Lahey. The iodine tolerance test as an aid in the diagnosis of clinical hyperthyroidism. New England J. Med. *216*: 501. 1937. *See also* The level of iodine in the blood. Arch. Int. Med. *65*: 882. 1940. 81, 94, 95, 98, 106, 250

434. Pfeiffer, G. Der gallen- und Schilddrüsenjodgehalt bei Rindern unter dem Einfluss jahreszeitlicher Futterumstellung. Biochem. Ztschr. *230*: 290. 1931. 204

435. Phillips, F. J., G. M. Curtis, and O. Erf. Results of prolonged increased iodine feeding. Proc. Soc. Exper. Biol. & Med. *31*: 585. 1934. 201

436. —— The effects of prolonged increased iodine feeding. Ohio J. Sc. *35*: 286. 1935. 202

437. Pick, E. P. Zur Kenntniss der peptischen Spaltungsprodukte des Fibrins. Ztschr. f. physiol. Chem. *28*: 219. 1899. 53

438. Pieper, W. Die Wirkung von thyreotroper Substanz des Hypophysenvorderlappens aud die entnervte Schilddrüse. Endokrinologie *14*: 8. 1934. 175

439. Pighini, G. Rapporti ormonali fra ipofisi, tiroide, e lo iodio del cervello. Biochem. e terap. sper. *22*: 607. 1935. Abstracted by A. E. Meyer in Chem. Abstr. *30*: 3871. 1936. 20, 177

440. Pincussen, L., and W. Roman. Über den Einfluss der Bestrahlung auf die Fraktionen des Jods und Broms im Tierkörper, besonders nach Zufuhr von Jodsalzen. Biochem. Ztschr. *216*: 336. 1929. 7

441. Plummer, H. S. Results of administering iodin to patients having exophthalmic goiter. J.A.M.A. *80*: 1955. 1923. 89, 112, 119, 258

442. Puppel, I. T., and G. M. Curtis. The iodine balance in nodular goiter. J. Clin. Investigation *17*: 729. 1938. 192

443. De Quervain, F., and W. E. Smith. The iodine content of blood in ordinary goitres and in cretinism. Endocrinology *12*: 177. 1928. 83, 207

444. Raab, W. Wirkungsweise jodfreier Thyroxinvorstufen bei Hyperthyreosen. Wien. Arch. f. inn. Med. *23*: 321. 1933. 111

445. Rabinowitch, I. M., and A. B. Frith. I. The avidity of the thyroid gland for various iodine compounds *in vitro*. J. Clin. Investigation *1*: 473. 1925. 103

446. Rabinowitch, J. The effect of feeding potassium iodide on the proliferative activity of the thyroid gland in guinea pigs. Am. J. Path. *4*: 601. 1928. 105, 106

447. Rabourdin, M. Essai sur le dosage de l'iode dans les substances organiques à l'aide du chloroforme. Compt. rend. Acad. d. sc. *31*: 784. 1850. 4

448. Rapport, D., and A. Canzanelli. A comparison of the effects of tyrosine, diiodotyrosine, diiodothyronine, and thyroxine upon metabolism. Am. J. Physiol. *97*: 553. 1931. 111, 112

449. Rasmussen, A. T. Reaction of the supraoptic nucleus to hypophysectomy. Proc. Soc. Exper. Biol. & Med. *36*: 729. 1937. *See also* Innervation of the hypophysis. Endocrinology *23*: 263. 1938. 171, 173

450. Rawson, R. W., and P. Starr. Direct measurement of height of thyroid epithelium; a method of assay of thyrotropic substance; clinical application. Arch. Int. Med. *61*: 726. 1938. *See also* R. W. Rawson, and W. T. Salter. Microhistometric assay of thyrotropic hormone in day-old chicks. Endocrinology *27*: 155. 1940. 286

451. Read, J. M. Studies in exophthalmic goiter. I. Its incidence throughout the United States. Arch. Int. Med. *63*: 71. 1939. 251

452. Redisch, W., and W. H. Perloff. The medical treatment of hyperthyroidism. Endocrinology *26*: 221. 1940. 261

453. Reijst-Schäfer, A. Der Übergang von Jodiden in die Milch. Pharm. Weekbl. *45*: 1359. 1908. Cited in Chem. Abstr. *3*: 661. 1909. 71

454. Reiss, M., K. A. Winter, and J. Valdecasas. Studien über die Funktion der Nebennierenrinde. VI. Die Wirkung des Nebennierenrindenhormons auf den respiratorischen Standardumsatz des Kaninchens. Endokrinologie *11*: 97. 1932. 142

455. Reith, J. F. Kropf und Jod in Holland. Schweiz. med. Wchnschr. *14*: 791. 1933. 188, 192

456. Remington, R. E., E. J. Coulson, and H. Levine. Studies on the relation of diet to goiter. III. Further observations on a goitrogenic diet. J. Nutrition *11*: 343. 1936. IV. The antigoitrogenic value of some foods. Ibid. *12*: 27. 1936. 123, 192

457. Rice, C. O. Histologic structure of the normal thyroid gland. Arch. Surg. *36*: 96. 1938. *See also* J. F. McClendon, and C. O. Rice. Blood hormone iodine and iodine reserve in relation to basal metabolic rate. Proc. Soc. Exper. Biol. & Med. *40*: 379. 1939. 122

458. Riddle, O., and J. Křížanecký. Studies on the physiology of reproduction in birds. XXVIII. Extirpation of thymus and bursa in pigeons with a consideration of the failure of thymectomy to reveal thymus function. Am. J. Physiol. *97*: 343. 1931. 157

459. Rienhoff, W. F., Jr. A new conception of some morbid changes occurring in diseases of the thyroid gland based on experimental studies of the normal gland and the thyroid in exophthalmic goiter. Medicine *10*: 257. 1931. 8

460. Roberts, A. Personal communication. Jan. 6, 1940. 218

461. Roberts, A., R. D. Evans, and S. Hertz. Personal communication. Jan. 6, 1940. 215

462. Roberts, A., and J. W. Irvine, Jr. Concentration of radiohalides, and failure to observe gamma-rays from I^{128}. Physical Rev. *53*: 609. 1938. 219

463. Ross, D. A., and R. S. Schwab. The cortical alpha rhythm in thyroid disorders. Endocrinology *25*: 75. 1939. 161

464. Rowe, A. W. Endocrine studies. XXXV. The association of hepatic dysfunction with thyroid failure. Endocrinology *17*: 1. 1933. 210

465. Saegesser, M. Mineralstoffalkalität und Jodgehalt von Kropfgewebe. Biochem. Ztschr. *254*: 475. 1932. 118

466. —— Schilddrüse, Jod und Kropf: Klinische und experimentelle Untersuchungen. Helvet. med. acta, supplementum IV. *6*: 1–163. 1939. 5

467. Salter, W. T. The enzymic synthesis from thyroid peptone of an artificial protein which relieves myxedema. Am. J. Physiol. *113*: 114. 1935. 62, 185

468. —— Fluctuations in body iodine. Physiol. Rev. *20*: 345. 1940. 109

469. Salter, W. T., and J. Lerman. The metabolic effects of human thyroglobulin and its proteolytic cleavage products. J. Clin. Investigation *14*: 691. 1935. 12, 30, 230

470. —— The genesis of thyroid protein: clinical assays of artificial thyroid protein in human myxedema. Endocrinology *20*: 801. 1936. 61, 63, 106, 118, 120, 121, 135

471. Salter, W. T., and J. Lerman. Iodinated protein in human athyreosis. Tr. A. Am. Physicians *53*: 202. 1938. 18, 52

472. Salter, W. T., J. Lerman, and J. H. Means. The calorigenic action of thyroxin polypeptide. J. Clin. Investigation *12*: 327. 1933. 18, 184, 185

473. —— The calorigenic action of d- and l-thyroxine. J. Clin. Investigation *14*: 37. 1935. 111, 116

474. Salter, W. T., and O. H. Pearson. The enzymic synthesis from thyroid diiodotyrosine peptone of an artificial protein which relieves myxoedema. J. Biol. Chem. *112*: 579. 1936. 63, 117

475. Scharrer, K. Die Jodanreicherung der Milch durch Jodfütterung und ihr Zusammenhang mit der Frage der Kropfprophylaxe. Ztschr. f. d. ges. exper. Med. *56*: 677. 1927. 201

476. —— Chemie und Biochemie des Jods. Stuttgart, F. Enke. 1928. 4, 193, 194

477. Scharrer, K., and J. Schwaibold. Zur Kenntnis des Jods als biogenes Element. VI. Mitt. Über den Chemismus des tierischen Jodstoffwechsels. Biochem. Ztschr. *180*: 307. 1927. 201

478. —— Zur Kenntnis des Jods als biogenes Element. XV. Mitt. Tierische Organe und Produkte und deren Jodgehalt. Biochem. Ztschr. *195*: 228. 1928. 71

479. Scheffer, L. Jodgehalt der Nahrung und Kropfhäufigkeit. Biochem. Ztschr. *247*: 418. 1932. 101

480. —— Über die Jodbilanz normaler Menschen. Biochem. Ztschr. *259*: 11. 1933. 67, 187, 188, 193, 194

481. —— Jodstoffwechsel bei Schilddrüsenkranken. Klin. Wchnschr. *12*: 1285. 1933. 197

482. —— Jodstoffwechsel bei Hyperthyreosen. Klin. Wchnschr. *13*: 1570. 1934. 187, 208, 210

483. —— Jodstoffwechsel bei Schilddrüsenkrankheiten. Ztschr. f. klin. Med. *132*: 343. 1937. 187, 193, 212

484. Scheffer, L., and L. v. Megay. Jodstoffwechsel bei Kropfträgern. Klin. Wchnschr. *14*: 1360. 1935. 187, 190, 212

485. Scheringer, W. Beitrag zur Kenntnis des Blutjodspiegels beim Weibe unter physiologischen Bedingungen. Arch. f. Gynäk. *143*: 319. 1930. *See also* O. Bokelmann, and W. Scheringer. Beitrag zur Kenntnis der Schilddrüsenfunktion und des Jodstoffwechsels in der Gestation. Ibid. p. 512. 151, 153

486. —— Ueber den Jodstoffwechsel des Weibes. Deutsche med. Wchnschr. *57*: 883. 1931. *See also* O. Bokelmann, and W. Scheringer. Der Einfluss der Kastration auf den Funktionszustand der Schilddrüse bei der weiblichen Albinoratte. Arch. f. Gynäk. *148*: 1. 1932. 154, 156

487. —— Die Jodausscheidung im Harn der Schwangeren unter physiologischen und experimentellen Bedingungen. Arch. f. Gynäk. *145*: 701. 1931. 154

488. —— Zur Frage der Hyperfunktion der Schilddrüse in der Gravidität: Über die Wirkung des Adrenalins auf den Blutjodspiegel der Schwangeren. Klin. Wchnschr. *12*: 107. 1933. 179

489. SCHITTENHELM, A., AND B. EISLER. Über die Resorption des Thyroxins nach oraler Zufuhr. Ztschr. f. d. ges. exper. Med. *80*: 569. 1932. 82, 184, 205

490. —— Therapeutische Versuche mit Thyreotropen Hormon. Klin. Wchnschr. *11*: 1783. 1932. 84, 128, 129

491. —— Der Blutjodspiegel in seiner pathologisch-physiologischen und klinischen Bedeutung. Klin. Wchnschr. *11*: 6. 1932. 91

492. —— Über die Wirkung des Adrenalins auf den Blutjodspiegel. *Also* Thyroxin und Zentralnervensystem. Klin. Wchnschr. *11*: 9. 1932. 93, 177

493. —— Über die Resorption des Thyroxins nach oraler Zufuhr. Ztschr. f. d. ges. exper. Med. *80*: 569. 1932. *See also* Über die Resorption und Ausscheidung des Thyroxins beim schilddrüsengesunden, beim schilddrüseninsuffizienten und beim hyperthyreotischen Menschen. Ibid. p. 580. 193, 203, 204, 208

494. —— Der Jodstoffwechsel des Myxödematösen. Ztschr. f. d. ges. exper. Med. *80*: 589. 1932. 180, 206, 207

495. —— Über die Verteilung des Jodes im Zentralnervensystem nach Zufuhr von Schilddrüsenstoffen. Ztschr. f. d. ges. exper. Med. *86*: 275. 1933. 20, 23, 176

496. —— Über die Verteilung des Jodes im Zentralnervensystem bei Mensch und Tier. Ztschr. f. d. ges. exper. Med. *86*: 290. 1933. 20

497. —— Über den Einfluss der Narkose auf den Jodgehalt des Blutes. Ztschr. f. d. ges. exper. Med. *86*: 368. 1933. 174, 205

498. SCHMELLING, J. W. Over de normale en vergroote Schildklier gedurende de embryonale ontwikkeling, bij den pasgeborene en bij het jonge kind in Nederland. Dissert. Utrecht. 1934. 154, 155, 191

499. SCHNEIDER, E., AND E. WIDMANN. Klinische und experimentelle Untersuchungen zum Problem des Kropfes und der Basedowschen Krankheit. I. Mitt. Untersuchungen zur Frage des Blutjodgehaltes. Deutsche Ztschr. f. Chir. *231*: 305. 1931. 68, 71

500. SCHOCKAERT, J. A., AND G. L. FOSTER. Influence of anterior pituitary substances on the total iodine content of the thyroid gland in the young duck. J. Biol. Chem. *95*: 89. 1932. 128

501. SCHOENHEIMER, R., AND D. RITTENBERG. Deuterium as an indicator in the study of intermediary metabolism. J. Biol. Chem. *111*: 163. 1935. 213

502. SEABORG, G. T., J. J. LIVINGOOD, AND J. W. KENNEDY. Radioactive tellurium: further production and separation of isomers. Physical Rev. *55*: 794. 1939. 216

503. SEGALL, H. N. Unpublished data. Thyroid Clinic, Massachusetts General Hospital. Cited by J. H. Means: The Thyroid and Its Diseases. Philadelphia, J. B. Lippincott. 1937. 107, 122, 252

504. Seidlin, S. M. The metabolism of the thyrotrophic and gonadotrophic hormones. Endocrinology *26*: 696. 1940. 98, 140

505. Sevringhaus, A. E. Cytological observations on secretion in normal and activated thyroids. Ztschr. f. Zellforsch. u. mikr. Anat. *19*: 653. 1933. 9, 106, 121

506. Shapiro, S., and D. Marine. Clinical report of a case of Graves' disease with rapid improvement following the oral administration of fresh ox suprarenal gland. Endocrinology *5*: 699. 1921. 142

507. Sherrington, C. S. The Integrative Action of the Nervous System. New Haven, Yale Univ. Press. 1906. 171

508. Siebert, W. J., and C. S. Linton. Comparison of the effects of potassium iodide and of diiodotyrosin upon basal metabolism. J. Pharmacol. & Exper. Therap. *53*: 418. 1935. 106, 122, 137

509. Siebert, W. J., and E. W. Thurston. The effects of combinations of KI with acid anterior pituitary extracts, KI with Armour's anterior pituitary, and KI with thyroid substance upon basal metabolism in guinea pigs. J. Pharmacol. & Exper. Therap. *46*: 293. 1932. 134

510. Silberberg, M. Effects of combined administration of extracts of anterior lobe of pituitary and of potassium iodide on thyroid gland. Proc. Soc. Exper. Biol. & Med. *27*: 166. 1929. *See also* Die Wirkung von Jodkalium und Extrakt des Vorderlappens der Hypophyse auf die Schilddrüse des Meerschweinchens. Krankheitsforschung *8*: 171. 1930. 133, 134

511. Smelser, G. K. Chick thyroid responses as a basis for thyrotropic hormone assay. Endocrinology *23*: 429. 1938. 287

512. Smith, H. W., W. Goldring, and H. Chasis. The measurement of the tubular excretory mass, effective blood flow, and filtration rate in the normal human kidney. J. Clin. Investigation *17*: 263. 1938. 64

513. Smith, I. H., and H. C. Moloy. The effect of nerve stimulation and nerve degeneration on the mitochondria and histology of the thyroid gland. Anat. Rec. *45*: 393. 1930. 167

514. Smith, P. E. The effect of hypophysectomy in the early embryo upon the growth and development of the frog. Anat. Rec. *11*: 57. 1916. 125

515. Snapper, I., and A. Grunbaum. Metabolism and action of diiodotyrosine and n-benzoyldiiodotyrosine. Brit. J. Exper. Path. *18*: 401. 1937. 183

516. Spence, A. W. Discussion of A. Loeser. Hyperthyroidism and the thyrotropic hormone of the pituitary. Brit. M. J. *1*: 1276. 1937. 141

517. Starr, P., and H. Patton. The effect of pregnancy urine extract and ovarian follicular hormone on hyperthyroidism. Endocrinology *18*: 113. 1934. *See also* Observations of remissions in hyperthyroidism induced by pregnancy urine extract. Ann. Int. Med. *8*: 825. 1935. 93, 158, 191

518. STARR, P., H. PATTON, AND R. C. BRUNER. The response of the thyroid of the guinea pig to anterior pituitary thyreotropic preparations and its relation to the functional state of the ovary. Med. Papers dedicated to Henry Asbury Christian, Baltimore. p. 392. 1936. 156

519. STARR, P., AND R. W. RAWSON. A graphic representation of thyroid response to stimulation by thyrotropic hormone. Proc. Soc. Exper. Biol. & Med. *35*: 603. 1937. 287

520. STARR, P., R. W. RAWSON, R. E. SMALLEY, E. DOTY, AND H. PATTON. The microhistometric method applied to thyrotropic hormone assay. West. J. Surg. *47*: 65: 1939. 145, 287

521. STEVENS, C. D. Determination of iodine in biological materials; a simplified technique. J. Lab. & Clin. Med. *22*: 1074. 1936–37. 271

522. STRAUB, J., AND G. PAPP. Über den Jodgehalt von Schilddrüsen aus der grossen ungarischen Tiefebene in Verbindung mit der Verbreitung des Kropfes. Endokrinologie *8*: 342. 1931. 123

523. STRAUSS, E., AND R. GRÜTZNER. Mitteilungen aus dem Gebiete der Eiweisschemie. II. Über Jodglobin. Ztschr. f. physiol. Chem. *112*: 167. 1920. 18

524. STURGIS, C. C., S. ZUBIRAN, G. W. WELLS, AND TH. BADGER. The effect of iodine by mouth on the reaction to intravenous injections of thyroxin. J. Clin. Investigation *2*: 289. 1926. 122

525. STURM, A. II. Mitt. Beitrag zur Kenntnis des Jodstoffwechsels der Hyperthyreosen. Ber. ü. den 39 Kongress d. Deutsch. ges. inn. Med. Wiesbaden, p. 93. 1927. 210

526. —— Beiträge zur Kenntnis des Jodstoffwechsels. III. Mitt. Der Jodstoffwechsel des schilddrüsenlosen Hundes. Deutsches Arch. f. klin. Med. *161*: 129. 1928. 84, 193, 201, 206

527. —— Beiträge zur Kenntnis des Jodstoffwechsels. V. Mitt. Stoffwechselstudien an den überlebenden Schilddrüse. I. Teil. Der Jodstoffwechsel der überlebenden künstlich durchströmten Hundeschilddrüse. Ztschr. f. d. ges. exper. Med. *74*: 514. 1930. 82, 103, 104, 106, 107, 180

528. —— Beiträge zur Kenntnis des Jodstoffwechsels. VII. Mitt. Einfluss des Zwischenhirns bzw. der Hypophyse auf den Jodstoffwechsel. Ztschr. f. d. ges. exper. Med. *93*: 490. 1934. 20, 126, 177, 193

529. STURM, A., AND B. BUCHHOLZ. Beiträge zur Kenntnis des Jodstoffwechsels. IV. Mitt. Jodverteilung im menschlichen und tierischen Organismus in ihrer Beziehung zur Schilddrüse. Deutsches Arch. f. klin. Med. *161*: 227. 1928. 6, 15, 19–22, 71, 125, 137, 138, 143, 148

530. STURM, A., AND R. SCHNEEBERG. Beiträge zur Kenntnis des Jodstoffwechsels. VI. Mitt. Das Gehirnjod. Ztschr. f. d. ges. exper. Med. *86*: 665. 1933. 20, 23, 137, 176, 177, 180

531. STURM, A., AND W. SCHÖNING. Nachweis des thyreotropen Hormons in nicht-hypophysären Geweben. Zugleich ein Beitrag zur Frage des Tropismus in der Endokrinologie. Endokrinologie *16*: 1. 1935. 126, 128

532. SVEDBERG, T. Personal communication. 1938. 37

533. Svedberg, T. Protein molecules. Chem. Rev. *20*: 81. 1937. 35, 37, 41

534. Szász, E. Über Jodausscheidung gesunder und hyperthyreoter Menschen im Höhenklima. Med. Klin. *29*: 1584. 1933. 67, 71, 210

535. Tape, G. F., and J. M. Cork. Induced radioactivity in tellurium. Physical Rev. *53*: 676. 1938. 213

536. Tatum, A. L. A study of the distribution of iodine between cells and colloid in the thyroid gland. I. Methods and results of study of beef, sheep, and pig thyroid glands. J. Biol. Chem. *42*: 47. 1920. 9

537. Thomas, H. M., Jr., and A. C. Woods. Progressive exophthalmos following thyroidectomy. Bull. Johns Hopkins Hosp. *59*: 99. 1936. 132

538. Thompson, W. O. Studies in blood volume. I. The blood volume in myxedema, with a comparison of plasma volume changes in myxedema and cardiac edema. J. Clin. Investigation *2*: 477. 1925–26. 116

539. Thompson, W. O., J. M. Alper, P. K. Thompson, and L. F. N. Dickie. The effect of diiodotyrosine on the basal metabolism in myxedema. J. Clin. Investigation *13*: 29. 1934. 110, 112

540. Thompson, W. O., A. C. Cohen, P. K. Thompson, E. G. Thorp, and A. G. Brailey. The range of effective iodine dosage in exophthalmic goiter. III. The effect on basal metabolism of the daily administration of one-quarter drop of compound solution of iodine and of slightly smaller doses, with a summary of results to date. Arch. Int. Med. *45*: 430. 1930. 121, 253, 256

541. Thompson, W. O., L. L. McLellan, P. K. Thompson, and L. F. N. Dickie. The rates of utilization of thyroxine and of desiccated thyroid in man: the relation between the iodine in desiccated thyroid and in thyroxine. J. Clin. Investigation *12*: 235. 1933. 116

542. Thompson, W. O., P. K. Thompson, A. G. Brailey, and A. C. Cohen. The calorigenetic action of thyroxin at different levels of basal metabolism in myxedema. J. Clin. Investigation *7*: 437. 1929. *See also* reference 541. 108

543. Thompson, W. O., P. K. Thompson, and A. C. Cohen. The range of effective iodine dosage in exophthalmic goiter. IV. The effect on basal metabolism of the daily administration of about 0.75 mg. of iodine. Arch. Int. Med. *49*: 199. 1932. *See also* W. O. Thompson, S. G. Taylor, III, P. K. Thompson, and L. F. N. Dickie. Quantitative observations on the effect of iodine in exophthalmic goiter in Chicago. West. J. Surg. *43*: 489. 1935. 121, 255

544. Thompson, W. O., P. K. Thompson, L. F. N. Dickie, and J. M. Alper. Effect of alkali on the absorption of thyroxine from the gastro-intestinal tract, with a note on the comparative effects of synthetic and "natural" thyroxine injected intravenously. Arch. Int. Med. *52*: 809. 1933. 52, 184, 203

545. Thompson, W. O., P. K. Thompson, S. G. Taylor, III, and L. F. N. Dickie. Calorigenic potency of free thyroxine by mouth. Endocrinology *24*: 87. 1939. *See also* W. O. Thompson, P. K. Thompson, S. G. Taylor, III, S. B. Nadler, and L. F. N. Dickie. The pharmacology of the thyroid in man. J.A.M.A. *104*: 972. 1935. 82, 116

546. Thompson, W. O., P. K. Thompson, S. G. Taylor, III, S. B. Nadler, and L. F. N. Dickie. Calorigenic action of extracts of the anterior lobe of the pituitary in man. Endocrinology *20*: 55. 1936. 128

547. Tiedcke, C. Experimentelle und Klinische Beiträge zur Pharmakologie des Jods. II. Mitt. Über die Resorption von Jod durch die Mundschleimhäute und seine Verteilung im Organismus. Klin. Wchnschr. *12*: 383. 1933. 100, 183

548. Timpe, O. Das Verhalten des Jodspiegels des Blutes während der chirurgischen Behandlung d. M. Basedow. Arch. f. klin. Chir. *187*: 104. 1936. 90

549. Trevorrow, V. Studies on the nature of the iodine in blood. J. Biol. Chem. *127*: 737. 1939. 18, 72–74, 88

550. Trevorrow, V., and G. J. Fashena. The determination of iodine in biological material. J. Biol. Chem. *110*: 29. 1935. 79, 218

551. Turner, K. B., A. DeLamater, and W. D. Province. Observations on the blood iodine. I. The blood iodine in health, in thyroid and cardiorenal disease, and in leukemia. J. Clin. Investigation *19*: 515. 1940. 95

552. Turner, R. G. Iodine content of human skim milk from goitrous and non-goitrous regions. Proc. Soc. Exper. Biol. & Med. *30*: 1401. 1933. *See also* R. G. Turner, and C. W. Mathews. The iodine content of blood in certain pathological conditions. Proc. Am. Soc. Biol. Chemists, J. Biol. Chem. *92*: lxxxviii. 1931. 70, 143

553. Tyndale, H. H., and L. Levin. Ovarian weight responses to menopause urine injections in normal, hypophysectomized, and hypophysectomized thyroxine-treated immature rats. Am. J. Physiol. *120*: 486. 1937. 158

554. Uhlenhuth, E. The effect of iodine and iodothyrin on the larvae of salamanders. III. The rôle of the iodine in the specific action of the thyroid hormone as tested in the metamorphosis of the axolotl larvae. Biol. Bull. *42*: 143. 1922. 9, 19, 111

555. Uotila, U. U. The rôle of cervical sympathetics in the regulation of thyroid and thyrotropic function. Endocrinology *25*: 63. 1939. *See also* The regulation of thyrotropic function by thyroxin after pituitary stalk section. Ibid. *26*: 129. 1940. 166, 168, 170

556. —— The effect of estrin on the anterior pituitary of male rats after pituitary stalk section. Endocrinology *26*: 123. 1940. 175

557. van Dyke, H. B. A study of the distribution of iodine between cells and colloid in the thyroid gland. II. Results of study of dog and human glands. J. Biol. Chem. *45*: 325. 1920–21. III. The effect of stimulation of the vago-sympathetic nerve on the distribution and concentration of iodine in the dog's thyroid gland. Am. J. Physiol. *56*: 168. 1921. IV. The distribution of iodine in the hyperplastic thyroid gland of the dog after the intravenous injection of iodine compounds. J. Biol. Chem. *54*: 11. 1922. 9, 11, 13, 119, 166

558. —— On the absorption by the hyperplastic thyroid gland of various forms of iodine introduced intravenously. J. Pharmacol. & Exper. Therap. *25*: 166. 1925. 106

559. van Dyke, H. B. The Physiology and Pharmacology of the Pituitary Body. Vol. II. Chicago, Univ. of Chicago Press. 1939. 132

560. Veil, W. H., and A. Sturm. Beiträge zur Kenntnis des Jodstoffwechsels. Deutsches Arch. f. klin. Med. *147*: 166. 1925. 74, 81, 82, 84, 87, 93, 101, 143, 154, 179, 180

561. Veil, W. H., and R. Sturm. Beiträge zur klinischen Pharmakologie. III. Geschichte der Jodtherapie. Deutsches Arch. f. klin. Med. *154*: 327. 1927. 269

562. Vogt, M. Zur Frage der nervösen Regulation der Schilddrüsentätigkeit. Arch. f. exper. Path. u. Pharmakol. *162*: 129. 1931. 168

563. Wallace, G. B., and B. B. Brodie. The distribution of administered iodide and thiocyanate in comparison with chloride, and their relation to body fluids. J. Pharmacol. & Exper. Therap. *61*: 397. 1937. *See also* The distribution of administered iodide and thiocyanate in comparison with chloride in pathological tissues, and their relation to body fluids. Ibid. p. 412. 68, 255

564. Wasteneys, H., and H. Borsook. The enzymatic synthesis of protein. Physiol. Rev. *10*: 110. 1930. 62

565. Watson, E. M. An iodine tolerance test for the investigation of thyroid function. Endocrinology *20*: 358. 1936. 266

566. —— The relation of the iodine tolerance to thyroid function. Endocrinology *22*: 528. 1938. 106

567. Watson, E. M., and A. S. Barber. An apparatus for microtitrations especially adapted for the estimation of blood iodine. J. Lab. & Clin. Med. *22*: 972. 1937. 275

568. Webster, B., D. Marine, and A. Cipra. The occurrence of seasonal variations in the goiter of rabbits produced by feeding cabbage. J. Exper. Med. *53*: 81. 1931. 122

569. Weech, A. A., and S. M. Ling. Nutritional edema. Observations on the relation of the serum proteins to the occurrence of edema and to the effect of certain inorganic salts. J. Clin. Investigation *10*: 869. 1931. 154

570. Weil, R. Zur Frage der Schilddrüsenwirkung des Jods. Klin. Wchnschr. *15*: 387. 1936. 121, 166

571. Welch, H. Cause and prevention of hairless pigs in the United States. Montana Agric. Coll. Exper. Station Circ. *71*: 37. 1917. 13

572. Werner, S. C. Prolonged injection of a thyrotropic extract without development of refractoriness. Proc. Soc. Exper. Biol. & Med. *34*: 390. 1936. 94

573. —— The thyrotropic hormone and the antihormone problem. Endocrinology *22*: 291. 1938. 129

574. Wheeler, H. L., and G. S. Jamieson. Synthesis of iodgorgoic acid. Am. Chem. J. *33*: 365. 1905. 4, 50, 51

575. Williams, R. G. Microscopic studies of living thyroid follicles implanted in transparent chambers installed in rabbit's ear. Am. J. Anat. *62*: 1. 1937. 9

576. Williamson, G. S., I. H. Pearse, and H. M. Cunnington. The two products of thyroid activity. J. Path. & Bact. *31*: 255. 1928. 8, 70

577. Wilson, L. B., and E. C. Kendall. The relationship of the pathological histology and the iodin compounds of the human thyroid. Am. J. M. Sc. *151*: 79. 1916. 12, 119

578. Winchester, C. F. Influence of thyroid on egg production. Endocrinology *24*: 697. 1939. 157

579. Wittkower, E., W. Scheringer, and E. Bay. Über affektivsomatische Veränderungen. VI. Mitt. Zur affektiven Beeinflussbarkeit des Blutjodspiegels. Klin. Wchnschr. *11*: 1186. 1932. 194

580. Wolf, O., and R. Greep. Histological study of thyroid gland of hypophysectomized rats exposed to cold. Proc. Soc. Exper. Biol. & Med. *36*: 856. 1937. 168

581. Wolfram, K., and K. Pollak. Leczenie wstrzasu pooperacyjnego w chorobie Basedowa dozylnym podawaniem jodu. Warsz. Czas. Lek. *15*: 435. 1938. 92

582. Wormall, A. The immunological specificity of chemically altered proteins. Halogenated and nitrated proteins. J. Exper. Med. *51*: 295. 1930. 46, 57

583. Zawadowsky, B. M. Zur Problemstellung der Physiologie und des Stoffwechsels der Hormone im Organismus. Endokrinologie *12*: 81. 1933. 18

584. Zawadowsky, B., and G. Asimoff. Zur Frage der Feststellung von Thyroxin im Organismus hyperthyreoidisierter Säugetiere. Pflüger's Arch. f. d. ges. Physiol. *216*: 65. 1927. 19, 23, 72, 205, 206

585. Zawadowsky, B. M., and Z. M. Perlmutter. Über das Schicksal des Thyroxins im Blute und in den Geweben der hyperthyreoidisierten Hühner. Arch. f. Entwcklngsmechn. d. Organ. *109*: 210. 1927. 19, 205

586. Zondek, H. Coma basedovicum. Klin. Wchnschr. *9*: 1999. 1930. 85

587. —— The Diseases of the Endocrine Glands. Third (English) Ed. Baltimore, William Wood and Company. 1936. 85

588. Zunz, E., and J. La Barre. Action de la substance thyréotrope d'origine antéhypophysaire sur la teneur du sang en thyroxine. Compt. rend. Soc. de biol. *118*: 1622. 1935. 129

Incidental References

Abderhalden, E. Fermentforsch. *1*: 47. 1916. 62

Abel, M. S. Am. J. M. Sc. *200*: 220. 1940. 289

Barkan, G., and W. Leistner. Klin. Wchnschr. *8*: 117. 1929. 68

Bennholdt-Thomsen, C., and M. Wellmann. Klin. Wchnschr. *13*: 800. 1934. 103

Block, P., Jr. J. Biol. Chem. *135*: 51. 1940. .... 61, 110

Blotner, H., and H. J. Perkin. Personal communication. 172

Blum, F., and R. Grützner. Ztschr. f. physiol. Chem. *92*: 360. 1914 106

Bruger, M. Personal communication. ... 79

Carpenter, M. D., and G. R. Sharpless. J. Nutrition. *13*: 235. 1937. ... 190

Chatin, A. Compt. rend. Acad. d. sc. *31*: 280. 1850. ... 189

Chidester, F. E. Science *2*: 436. 1932. ... 190

Coons, A. H. Personal communication. ... 96–98, 114, 241, 284

Coplan, H. M., and M. M. Sampson. J. Nutrition *9*: 469. 1935. ... 191

Corson, D. R., K. R. Mackenzie, and E. Segrè. Personal communication. *See also* Physical Rev. *57*: 1087. 1940. ... 234

Crabtree, M. G., and E. M. Mason. *See* Young, Crabtree and Mason. ... 189

Curie, Pierre (Mme.). Radioactivité. Paris, Hermann & Cie. 1936. ... 213

Curtis, G. M. *See* Ref. 112. ... 250

Curtis, G. M., and F. J. Phillips. *See* Ref. 114. ... 194

Dandy, W. E. J.A.M.A. *114*: 312. 1940. ... 172

Davis, C. H. Am. J. Obst. & Gynec. *24*: 607. 1932. ... 191

Davis, J. E., and A. B. Hastings. Am. J. Physiol. *114*: 618. 1936. ... 115

Elliott, T. R. J. Physiol. *44*: 374. 1912. ... 171

Elmer, A. W., and Salzer. Cited by Elmer, Ref. 139. ... 16

Evans, H. M. Ann. Rev. Physiol. *1*: 577. 1939. ... 234

Fasold, H., and H. Peters. Ztschr. f. d. ges. exper. Med. *92*: 57. 1934. ... 190

Fellenberg, Th. von. *See* Ref. 167. ... 181

Fellenberg, Th. von. Biochem. Ztschr. *152*: 141. 1924. ... 189

Fenger, F. J. Biol. Chem. *12*: 55. 1912. *Ibid.* *29*: 695. 1915. ... 14

Fermi, E. Rend. ist. sanita Pubblica *1*: 421. 1938. ... 213

Fevold, H. L., M. O. Lee, F. L. Hisaw, and E. J. Cohn. Endocrinology *26*: 999. 1940. ... 126

Fisher, C. *See* Ref. 351. ... 171

Goormaghtigh, W., and H. Handovsky. Compt. rend. Soc. de biol. *218*: 1616. 1935. ... 103

Gray, S. H., and L. Loeb. Am. J. Path. *4*: 257. 1928. ... 106

Gray, S. H., and J. Rabinowitch. Am. J. Path. *5*: 485. 1929. ... 107

Gutman, A. B., E. N. Benedict, and W. W. Palmer. Proc. Soc. Exper. Biol. & Med. *29*: 1198. 1932. ... 25

Hair, G. W. *See* Ref. 215. ... 171

Hamilton, J. G. Personal communication. ... 234

Hare, K. Am. J. Physiol. *119*: 326. 1937. ... 171

Hektoen, L., and K. Schulhof. Proc. Nat. Acad. Sc. *11*: 481. 1925. ... 14

Hellwig, C. A. Surg., Gynec. & Obst. *64*: 604. 1937. Arch. Surg. *40*: 98. 1940. ... 192, 251

Hercus, C. E., W. N. Benson, and C. L. Carter. J. Hyg. *24*: 321. 1925. ... 189

HERTZ, S. Personal communication. 260

HERTZ, S., AND A. KRANES. *See* Fig. 15. 127

HERZFELD, E., AND R. KLINGER. Schweiz. med. Wchnschr. *3*: 724. 1922. 16

HOGBEN, L. T., AND F. A. E. CREW. Brit. J. Exper. Biol. *1*: 1. 1923. 14

INGRAM, W. R., C. FISHER, AND S. W. RANSON. Arch. Int. Med. *57*: 1067. 1936. 171

JANSEN, W. H., AND F. ROBERT. Deutsches Arch. f. klin. Med. *157*: 224. 1927. 16

JENSEN, H., AND S. TOLKSDORF. J. Biol. Chem., Proc. *133*: xlix. 1940. 126

JOLIOT, F., AND I. CURIE. Nature *133*: 201. 1934. 213

KIMBALL, O. P. *See* Ref. 280. 254

KOCHER, T. Arch. f. klin. Chir. *92*: 1166. 1910. 130

KRAUSS, W. E., AND C. F. MONROE. J. Biol. Chem. *89*: 581. 1930. . . 191

KUSCHINSKY, G. *See* Table 30. 132

LAWRENCE, E. O. *See* Ref. 308. 234

LELKES, Z. Endokrinologie *13*: 35. 1933. 14

MAGOUN, H. W. *See* Ref. 351. 171

MAZZOCCO, P. Compt. rend. Soc. de biol. *112*: 867. 1929. 189

MCCARRISON, R. Brit. M. J. *1*: 966. 1931. 192

MCCLENDON, J. F., W. C. FOSTER, AND W. G. KIRKLAND. J. Biol. Chem. (Scient. Proc.) *133*: xliv. 1940. 86

MELLANBY, E., AND M. MELLANBY. J. Physiol. (Proc.) *55*: vii. 1921. 190

MISLOWITZER, E., R. NISSEN, AND L. STANOYÉVITCH. Compt. rend. Soc. de biol. *115*: 885. 1934. . . . . 179, 180

MITSUI, K. Tr. Jap. Path. Soc. *20*: 267. 1930. Abstracted in Ber. Physiol. *60*: 109. 1931. 102

MUUS, J. M. Personal communication. 98, 114, 275, 284

NITSCHKE, A. Klin. Wchnschr. *12*: 1793. 1933. 103

OKKELS, H. J. Exper. Med. *66*: 297, 305. 1937. 129

OSWALD, A. Virchows Arch. f. path. Anat. *169*: 444. 1902. 16

PERKIN, H. J. *See* Ref. 433. 250

PERKIN, H. J., AND R. B. CATTELL. Surg., Gynec. & Obst. *68*: 745. 1939. 259

PETERS, J. P. Body Water, the Exchange of Fluids in Man. Thomas, Springfield. 1935. 68

PETERS, J. P., AND D. D. VAN SLYKE. Quantitative Clinical Chemistry, Vol. II, Methods. Baltimore, Williams & Wilkins Co. 1932. . . . . . 278, 279

PITTMAN, H. S. New England J. Med. *210*: 912. 1934. 260

PLINY THE ELDER (CAIUS PLINIUS SECUNDUS). Historiae naturalis. lib. xxxvii, cap. 11 (2). 188

PUPPEL, I. D., AND G. M. CURTIS. Arch. Path. *26*: 1111. 1938. . . . . 197, 198

RAAB, W. Wien. med. Wchnschr. *82*: 1549. 1932. 251

RANSON, S. W. Psychosom. Med. *1*: 486. 1939. 171–173

Rehberg, P. B. Biochem. J. *19*: 270. 1925. 278

Riggs, D. S., and E. B. Man. *See* Ref. 370. 276

Rush, B. Med. & Physical J. *16*: 193. 1806. 146

Salter, W. T. Proc. Soc. Exper. Biol. & Med. *24*: 116. 1926. 35

Sappington, T. S. Personal communication. 284

Scheffer, L. *See* Ref. 481 and 482. 190, 208

Schneider, E. Tr. Third Internat. Goiter Conf. and Am. A. Study Goiter. 1938. p. 439. 190

Schoeller, W. *See* Ref. 266. 128, 134

Selye, H. Canad. M. A. J. *34*: 706. 1936. 144

Simmons, S. M. Personal communication. 76, 96, 97, 240

Smith, G. E. J. Biol. Chem. *29*: 215. 1917. 14

Stellar, L. I., and H. G. Olken. Endocrinology *27*: 614. 1940. 99

Suk, V. Anthropologie, Praha. *9*: 1. 1931. 191

Sweet, J. F., and J. W. Ellis. J. Exper. Med. *22*: 732. 1915. 143

Taksaugi, T. Proc. 27th Annual Meeting Jap. Soc. Int. Med. Jap. J. M. Sc. Section VIII. Internal Medicine, Pediatry and Psychiatry *2*: 26*. 1931. 190

Tanabe, H. Beitr. z. path. Anat. u. z. allg. Path. *73*: 415. 1925. 192

Toryu, Y. Science Repts. Tôhoku Imp. Univ., 4th series. *8*: 107. 1933. 32

Veil, W. H. München. med. Wchnschr. *72*: 636. 1926. 103

Vogel. Gelehrte Anz. k. Bayerischen Akad. Wissensch. *27*: 223. 1848. Cited also by K. Sugimoto, J. Biol. Chem. *76*: 723. 1928. 31

Wheeler, H. L., and L. B. Mendel. J. Biol. Chem. *7*: 1. 1909–10 31

Whitaker, L. R., and G. Milliken. Surg., Gynec. & Obst. *40*: 17. 1925. 66

White, J. C. The Autonomic Nervous System. Revised second ed. Macmillan Co. 1940–41 164

Williams, J. W. *See* H. P. Lundgren. Nature *143*: 896. 1939. 37

Wislocki, G. B. Anat. Rec. *72*: 149. 1938. 163, 173

Young, M., M. G. Crabtree, and E. M. Mason. Med. Research Council (Britain). Special Rept. Series 271. 1936. 189

SUBJECT INDEX

SUBJECT INDEX

Acetylcholine, and parasympathetic stimulation, 171; and thyroid circulation, 168
Acromegaly, iodide therapy in, 122
Actinomycosis, iodine therapy in, 268
Addison's crisis, *see* Myxedema
Adrenal cortex, 20; antagonism to thyroid, 142; and gonads, 3; and iodine, 142; iodine in, 20; and ovary, 158; thyroxine in, 20; weight of, 20
Adrenal medulla, 143, 179; iodine in, 20; and thyroid, 143; thyroxine in, 20
Adrenals, and iodine balance, 125, 179; iodide and chloride in, 69; iodine after thyroidectomy, 137; iodine content of, 21, 151
Adrenine, effect on blood iodine, 179; effect on isolated thyroid gland, 180; synergism with splanchnic nerves, 171; synergism with thyrotropic hormone, 167; use in hyperthyroidism, 93
Adrenotropic hormone, and iodine, 142
Air, iodine in, 67, 187
Alps, goiter in, 189; iodine in air, 189
Amblystoma, 19
Amenorrhea, 157; and gonadotropic hormone, 175; effect on blood iodine, 152
Amphibia, thyroid in, 8
Anterior commissure, 164
Anterior pituitary, *see* Pituitary, anterior lobe
Antidiuretic hormone, 172
Antihormone, 93
Antimony, source of radioactive iodine, 215
Antithyroid substance, 93
Antithyrotropic substance, 94
Anxiety neurosis, case of, 241
Argentine, iodine in soil, 189
Arteriosclerosis, iodine therapy in, 269
Arteriovenous anastomosis, thyroidal, 166
Athyreosis, human, 60, 108, 109, 114, 117
Atomic weight, *see* Iodine
Atoms, radioactive, 213; disintegration of, 214
Atropine, effect on blood iodine, 179
Auricular fibrillation, 243, 245
Axolotl, metamorphosis of, 19

Barbiturates, 247; "luminal," 176; "prominal," 174, 176
Basal metabolism, 60, 78, 86, 88, 108, 109; and adrenal cortex, 142; and hypophysectomy, 126; effect of theelin, 158, 159; falsely increased, 78, 242, 244, 248; in simple goiter, 124
Beriberi, 190
Bern, goiter in, 124, 251; size of follicle, 251
Beta-ray, 214; emission from 8-day iodine, 218
Bile, after desiccated thyroid, 205; iodine content of, 151, 202, 204
Bio-assay, of colloid, 8; method of, 19
Biological iodine studies, history of, 4
Bismuth, transmutation to element 85, 234
Blastinomycosis, iodine therapy, 268
Blood, distribution of iodine and thiocyanate in, 68
Blood iodine, after adrenine, 179, 180; after hypophysectomy, 126; after iodide feeding, 72, 99, 151, 214, 219, 268; after surgical operations, 194; after thoracoplasty, 194; after thyroidectomy, 180; alcohol soluble, 73; and adrenal cortex, 142; "anorganische," 74; bio-assay of, 72; effect of climacteric, 72; effect of diet, 71; distribution between cells and plasma, 72; effect of drugs, 179; and exercise, 79, 80, 188; extraction of, 73; effect of hypothalamic narcosis, 174; after removal of hypothalamus, 179; hypothyroidism, 82, 239; in erythrocytes, 69, 72, 76, 255; in exophthalmic goiter, 71; lipoid bound, 74; effect on nervous activity, 179; "organische," 74; effect of ovarian cyclic activity, 151; effect of ovariectomy, 153, 155; "P" fraction, 243–248, 250; plasma, 76; precipitability, 73; and pregnancy, 155; prob-

lems, 94 ("D" and "T" fractions, 96–97; dialysis of, 99); protein bound, 74; qualitative nature, 72; quotient, effect of barbiturates, 174; seasonal variations, 72; serum, 72; effect of sex, 149; silver precipitate, 74; "T" fraction, 239, 241; effect of thyroidectomy plus ovariectomy, 150; total, 78–80, 87, 95; effect of tuber cinereum, 176; ultrafilterable, 72; whole blood, 76

Fasting, "anorganische," 79, 80; "organische," 79, 80; effect of exercise, 79; euthyroid, 78, 86; hyperthyroid, 78, 84, 86, 244, 245, 259; hypothyroid, 78, 83, 86, 246, 247; iodine quotient, 79; normal range, 79, 95; sex difference, 79; thyroid storm, 78, 247

Fluctuations in, 81; after iodide administration, 81, 97, 99, 256, 259, 266; after thyroxine derivatives, 82

Fractions of, 74, 239, 241; in exophthalmic goiter, 80, 258; in myxedema, 80; in nodular goiter, 80; diiodotyrosine fraction "D," 6, 74, 75, 239; inorganic iodide "I," 6, 75; protein precipitable "P," 75; thyroxine fraction "T," 6, 74, 75

Hyperthyroidism and, 73, 82, 85, 95, 239, 258; after adrenine, 93; after antihormones, 93; after assimilation of iodine compounds, 82, 89; after barbiturate therapy, 92; after hypothalamic narcosis, 176; after ovarian hormone, 93; after radiation therapy, 91; after surgical therapy, 90; after thyrotropic hormone, 84; after thyroxine, 84; blood thyroxine, 88; in thyroid storm, 91; iodine quotient, 88; relation to basal metabolic rate, 86

Body fluids, iodide in, 7, 69, 255

Boston, blood iodine in, 79

Brain, iodine in, 19, 21, 68, 138, 178; thiocyanate in, 68

Brain tissue, damage of, 176; effect of fructose, glucose, lactate, succinate, 161; effect of thyroid, carbohydrate metabolism of, 160, 161

Brain waves, and thyroid, 161

Bromide and iodides in epilepsy, 162

Bromine, reaction with iodide, 290

Buccal mucosa, *see* Mucous membrane

Cabbage goiter, 140, 191, 220, 223, 226; and iodothyrotoxicosis, 122

"Cachexia strumipriva," 14, 247, 269

Calcium metabolism, and parathyroid, 143; effect of iodine on, 191, 192

Calorigenic activity vs. metamorphosis, 112

Campen, goiter rate of, 188; iodine in water, 189

Cancer, prevention by thyrotropic hormone, 156; production by theelin, 157

Of thyroid, protein in, 13; iodine in, 223, 229, 230

Cardio-accelerating effect of thyroid preparations, 114, 116

Carotene, and thyroid activity, 190

Carotid plexus, 165

Carpathians, goiter in, 191

Casein, iodinated, 7, 51, 52, 61, 110; in human myxedema, 18

Castration, relation to hyperthyroidism, hypothyroidism, 159

"Castration phenomenon," 145

Cerebellum, iodine content of, 177, 178

Cerebrospinal fluid, after administration of iodide, 67; iodine in, 67, 219

Cervical sympathetic nerves to pituitary, 163, 169

Chichibu, goiter in, 189

Chloride, in lymph, 68, 70

Cholecystography, 66, 205

Choline, effect on blood iodine, 179

Circle of Willis, 173

Cleveland, goiter prophylaxis in, 192

Clinical metabolic iodine studies, 181, 194–200; problems, 237

Colloid, "inactive," 8; in exophthalmic goiter, 25, 120. *See also* Thyroid gland (colloid)

Colloid goiter, effect of iodide administration, 222; iodine in, 210, 211, 229–232; metabolism of radioactive iodine, 227; thyroglobulin in, 42

Colon, iodine in, 21

Colostrum, iodine in, 70

"Concentration coefficient," for thyroid iodine, 220, 221

"Corals," *see* Sea fans

Cortical alpha-rhythm, 161
Cretinism, 188; blood iodine fractions, 83, 86, 239; labeled iodine in thyroid, 232. *See also* Hypothyroidism
Cretinoid mothers, 13
Cyanide, 191; methyl, production of goiter, 223, 226
Cyclotron, in biological studies, 236; production of element 85, 234; production of radioactive iodine, 215, 218
Cystic fluid, 70

"D" iodine fraction, 6; in iodoprotein, 57; in thyroid, 17, 25; in thyroid protein, 29; in tissues, 18
Dermatitis, iodism of, 201
Desiccated thyroid, *see* Thyroid preparations
Deuteron, production of radioactive iodine, 215
Diabetes, *see* Pancreas
Diabetes insipidus, after brain injury, 172, 174; anatomical mechanism of, 164; urinary iodine in, 172
Diarrhea, and fecal iodine, 193, 202; "thyroidal diarrhea," 130
Dibromthyronine, 112
Dibromtyrosine, 112
Dichlordiiodothyronine, 112
Diencephalon, and endocrine balance, 125. *See also* Hypothalamus
Difluortyrosine, 112
Digitalis, 179
Diiodohistidine, 55
Diiodo-l-tyrosine, 4
Diiodothyronine, 59, 110, 112; in thyroid gland, 7; iodine content of, 290; molecular weight of, 290
Diiodotyrosine, 6, 7; action of trypsin on, 184; assimilation by mouth, 183; and bacteria, 184; calorigenic potency, 112; conversion to 3,5, diiodo-4 hydroxyphenyl lactic acid, 183; conversion to iodide, in saliva, 183; derivatives of, 115; and digestive enzymes, 183; dissociation of hydroxyl group, 28, 47; effect in exophthalmic goiter, 112; in bile, intestine, 184; in feces, 183, 201, 203; in intestine, 7; in urine, 183, 201; inhibition of thyrotropic hormone, 112; iodine content of, 51, 290; Kendall-Osterberg reaction, 53, 183; levorotatory, 183; excretion by liver, 203–205; metabolism of, 202–205; molecular weight of, 290; peptide of, 115; precursor of thyroxine, 112; properties of, 51; racemic, 183; storage of, 23; subcutaneous administration of, 183, 184
Diiodotyrosyl-thyroxine, 115
Diodrast, 64, 65

Echinus esculentus, eggs of, 22; ovaries of, 150; total iodine and "T" iodine of ovary, 150
Effingen, goiter rate of, 188, 189; iodine in soil and water, 188, 189
Egg albumin, iodinated, 55; combination with bromine and iodine, 27
Egg laying, and gonadotropic hormone, 175; and thyroid, 157
Eight-day iodine, 213–216, 220, 227
Eka-iodine, *see* Element 85
Electrodialyzable iodine, 7
Electro-encephalogram, and thyroid, 161
Electrolytes and water, distribution between cells and colloid, 11
Electrometer, DuBridge vacuum tube, 218
Electron, emission of, 214
Electroscope, Lauritsen quartz fiber, 217
Element 85, in blood, urine and feces, 235; in thyroid hyperplasia, 234; in thyrotoxic gland, 235, 236; in tissues, 235; preparation from bismuth, 234; trapping by thyroid gland, 234–236
Emotion, and hypothalamus, 163
Endemic goiter, etiology of, 123, 131, 147, 188, 191, 192
"Endocretion," 106, 120
Endocrine balance, 125, 145; and hypophysectomy, 126
Endocrine glands, and iodine balance, 141; iodine in, 6
Endocrine imbalance, and hypothalamus, 172; thyroid medication in, 252
Enzymes, and thyroid, 161; effect on thyroglobulin, 12; proteolytic, 12, 27
Ephedrine, effect on thyroid circulation, 168
Epilepsy, effect of iodide, 162
Epiphysis, iodine in, 21
Erepsin, digestion of thyroglobulin, 30

Erythrocytes, iodide in, 68, 255; "P" iodine in, 76, 96; total iodine in, 72
"Escape" from iodine, 120, 255, 256
Estrus, effect on thyroid iodine, 155
Ethyl iodide, inhalation of, 100
Evolution of thyroid, 8; of thyroxine, 7, 8
Excitement, and blood iodine, 242; and iodine excretion, 194
Exercise, and iodine excretion, 194; and iodine requirement, 188
Exophthalmic goiter, 118; and adrenal cortex, 142; circulating hormone, 77, 80, 86, 92, 95, 120, 258; diiodotyrosine in, 122; diiodotyrosine vs. iodine, 122; logarithmic fall in metabolism, 108, 120; hormonal activity in, 165; in boys, girls, 147; neural activity, 160, 165; relative iodine lack, 12, 96, 208; and sex, 147; thyroid block in, 120; thyroglobulin in, 42, 61; thyrotropic hormone, 129, 131, 139, 140
  Iodine therapy in, 119, 256, 264; effect of, 120, 121, 226, 231, 256, 267
Exophthalmos, after Langley operation, 167; after thyrotropic hormone, 127; malignant, orbital decompression in, 260
Extracellular fluids, chloride, iodide, thiocyanate in, 69

Fats, unsaturated, and goiter, 190; lipoidal iodine in blood, 74; lipoidal iodine in thyroid, 24, 101
Feces, diiodotyrosine in, 203; iodine content of, 151, 187, 188, 193 (from bile), 194; thyroxine in, 184, 193, 203. *See also* Iodine, excretion
Fetal thyroid, iodine therapy, 13; human, 14; hyperplasia of, 14, 191; of pig, sheep, 14. *See also* Newborn
Fever, and iodine excretion, 194
Fingernails, iodine in, 21
Fluorine, 192; and iodine metabolism, 191
Follicular cells, *see* Thyroid gland
Food, iodine in, 11, 185–189
Four-day iodine, 214
France, iodine in soil, 189

Gall bladder, iodine in, 205; function test, 66
Gamma, *see* Microgram
Gamma-ray, emission from 8-day iodine, 218, 233
Ganglion nodosum, 165
Geiger-Müller counter, 214, 219; accuracy of, 218
Globin, iodination of, 28, 49
Goiter, 123; colloid of, 12, 42; due to faulty intestinal absorption, 190; in boys, girls, 147, 192; in lactation, 191; in menopause, 191; in mountains, 189; in North America, 251; in pregnancy, 191; in puberty, 191; iodine in, 12, 123; iodine supply in food, water, 11, 188; iodine therapy, 12, 253; metabolism of radioactive iodine, 221–231; prophylaxis of, 147, 253; size of thyroid follicle, 251. *See also* Colloid goiter; Exophthalmic goiter; Hyperthyroidism; Lymphadenomatous goiter; Nodular goiter; Simple goiter; Struma lymphomatosa
Goitrogenic diet, 191, 226
Gonadotropic hormone, 128, 145, 154, 158, 174
Gonads, and adrenal cortex, 3, 156
Graves' disease, content of medulla oblongata in, 180; endocrine effects of, 160; exophthalmos, 238, 260; hypothalamic narcosis, 174, 176; and hypothalamus, 174; nervous phenomena, 160, 194, 237, 238, 250; neuro-endocrine relationships, 160, 171; pituitary-thyroid action, 144, 160; pulse rate, 238, 264; reaction to temperature, 238; weight loss, 238; without hyperthyroidism, 85, 96, 237, 250; without palpable goiter, 245. *See also* Exophthalmic goiter
Groák methods, 271; procedure I, 273; procedure II, 275
Growth, iodine requirement for, 186
Gumma, iodine therapy, 268

Hair, iodine in, 21
Hairless pigs, 13
Heart, iodine in, 21
Heart disease, arteriosclerotic, blood iodine in, 242, 245; hypertensive, 87, 242
Heart muscle, iodine content of, 151
Heat regulation, and exophthalmic goiter, 174; and posterior hypothalamus, 174; and supra-optic nucleus, 164

Helium ion, production of radioactive iodine, 215

Hetero-albumose, iodination of, 53

Hibernation, effect on thyroid gland, 103

Hippuran, 64, 65

Histidine, iodination of, 28, 50

Homothyroxine, 18, 61, 114

"Hormone" iodine, 73, 77

Hormones, *see* Thyroid hormone; Thyrotropic hormone

Hornussen, goiter rate, iodine in soil, 189

Hunter-Kendall estimation of iodine, 271

Hydrogen, heavy, 213

Hydroxy-thyroxine, 115

Hyperactive thyroid, *see* Hyperthyroidism

Hyperplastic thyroid tissue, 8; fluid and electrolyte in, 127

Hypertension, iodine quotient in, 87

Hyperthyroidism, amenorrhea in, 157; and adrenal cortex, 142; blood fractions, "P," "T," "I," 74, 76, 80, 84, 86, 96, 239, 240; blood iodine in, 78, 86, 95, 248, 259; blood iodine quotient in, 88; case of recurrent, 244, 251, 259; circulating hormone in, 108, 120; cortical alpha-rhythm in, 161; "D" fraction in thyroid, 17, 25, 120; deposition of colloid, 120; trapping of diiodotyrosine, 122; effect of iodine therapy, 17, 77, 120, 121, 256; effect of menstruation, 208; effect of pregnancy urine, 158; effect of theelin, 158; fecal iodine, 187, 198, 240; and goiter, 17, 119; without goiter, 245, 259, 260; inorganic iodide in blood, 17, 74, 77, 258; inorganic iodide in thyroid, 120; iodine in gland, 17, 118; iodine metabolism in, 200; iodine requirement in, 187, 198; liver disease in, 210; logarithmic fall in metabolism, 108, 120; peristalsis in, 161; polarity of cells, 121; reaction time in, 161; synthesis of thyroxine, 25, 63, 120; "T" fraction, 17, 120; and thymus, 144; thyroid block in, 120, 255; total thyroid iodine, 120, 210; urinary iodine, 198, 210, 211, 240; vasomotor nerves in, 161; and vitamins, 103, 190, 191

  Iodide therapy in, 17, 120, 122, 229–232, 253, 258; diagnostic therapeutic test, 253, 259, 263, 264

  Iodine stores in, 118; bio-assay of, 119; colloid reserves in, 118; "D" fraction, 17, 119; distribution of iodine, cells vs. colloid, 10, 119; effect of clinical exacerbation and remission, 119; effect of hyperplasia, 119; effect of iodine therapy, 17, 119; Graves' disease, 118; "T" fraction, 17, 119; thyroid protein in, 44, 118; thyroidal "diarrhea," 90, 118; toxic nodular goiter, 118, 229–232, 251, 267

  X-ray therapy in, 91, 261; blood iodine after, 262; thyroid storm after, 261

Hypoderm, iodine in, 21

Hypophysectomy, effects of, 126, 158, 168, 177

Hypophysial hegemony, 3

Hypophysis, *see* Pituitary

Hypothalamico-hypophysial nerve tracts, lesion of, 170, 172, 176; site of, 163

Hypothalamus, after anterior pituitary injection, 177; and gonadotropic hormone, 175; anesthesia of, 174, 176; damage of, 172, 176; iodine in, 20, 138, 176; and pituitary-thyrotropic axis, 171, 176; supraoptico-hypophysial tract, 164, 172; and sympathetic nervous system, 163, 164, 171; and water balance, 172

Hypothyroidism, after hypophysectomy, 126; blood iodine fractions, 76, 83, 86, 239, 248; cases of, 246; cortical alpha-rhythm, 161; cretinism, 13, 113, 118, 188; myxedema, 18, 60, 118, 207, 247; peristalsis in, 161; reaction time in, 161; relief by iodinated proteins, 113; thyroid parenchyma in, 118; thyrotropic hormone, in blood and urine, 133; vasomotor nerves in, 161. *See also* Myxedema; Cretinism

Hunzenschwil, goiter rate of, 188, 189; iodine in soil, 189; iodine in water, 188

I^{128}, 215, 216, 220

I^{131}, 213–216, 220, 227

Imidazole group, iodination of, 28

Immunity, 57; to iodoprotein, 57; to thyroglobulin, 75, 99, 129; to thyrotropic hormone, 93, 129

Infections, and iodine deficiency, 191, 194, 254

Insulin, 143; effect after pituitary stalk section, 173

Intestine, absorption of iodine compounds by, 182–185, 190; enzymes of, effect on thyroglobulin, 185; iodine content of, 21, 151; iodine excretion by, 202; parasites and iodine, 190
Iodase, 106
Iodate, assimilation of, 182; of potassium, iodine in, 290
Iodide, 6; absorption from buccal mucosa, 183; after iodide feeding, effect of sex, 149; dialyzable, 7; effect on colloid goiter, 12; effect on exophthalmic goiter, 12, 119; in expired air, 23; in lungs, 23; in lymph, 68; intestinal absorption of, 22, 182; placental circulation of, 13; retention of, 23.
Distribution of, 22, 255; in brain, 255; in thyroid, 103, 104; in tissues, 23, 255
See also Potassium iodide
Iodimetry, volumetric equivalents, 291
Iodine, after thyroidectomy, 201; age and thyroid concentration, 16; amniotic fluid, 13; anterior pituitary, 6, 19, 21, 138, 176; atomic weight, 290; balance, 181; circulating, 67; compound solution, 291; compounds of biological importance, 27; types of compound, 6; constants and factors, 290, 291; daily maintenance requirement, 181, 185; excretion after desiccated thyroid, 205, 206; diiodotyrosine-like iodine "D," 6; discovery of, in thyroid, 4, 27, 31; distribution of, in human goiters, 9; distribution of, in human thyroids, 10; dosage of, 238, 253; dosage in exophthalmic goiter, 121, 254; efficacy of, 254; electrodialyzable, 7; elementary, assimilation of, 182; and endocrine balance, 3, 125; estimation of, 270–286; excretion after brain lesions, 176; hyperactive thyroid, dose required, 121, 254; and infection, 191, 254; "iodine debauch," 269; in adrenal cortex, 6, 20, 21; in body reserves, 181; in body tissues, 6–26; in cartilage, 229; in cells, 6; in endocrine glands, 6; in epiphysis, 6; in excreta, 181, 192; in gonads, 20, 22, 151; in hay, 188; in ingesta, 181; in liver, 21, 68, 204, 229; in lymph, 68, 70; in medication, 181, 238, 254; relative lack, 191, 224, 254; studies, biological history of, 4; "Jodbasedow" from, 254, 256; Lugol's solution, 291; lymph nodes, 229; molecular weight, 290; muscle iodine, 6, 21, 229; organically bound, 6; and ovarian cycle, 148, 152; in ovary, 6, 21, 148, 149; parathyroid, 6, 21; partition of, in normal thyroids, 15, 17; and pregnancy, 153, 155, 191, 254; properties of, 290; prophylactic use of, 191, 253 (dosage, 192); quotient, 79, 87, 92, 258; in skeletal muscle, 6, 21, 229; standard solution of, 291; storage, 22 (by thyroid, 8, 100, 103, 104, 122); stores, 6; tincture of, 291; in tissues, 6–26, 229; therapy, 122, 238, 253, 254, 269; turnover, regulation of, 104; in urine of newborn, 13; and water pollution, 192, 254
Assimilation, of compounds, 182–184; of artificial thyroid protein, 185; of diiodotyrosine, 183; of labeled iodide, 182, 217; of organic iodine compounds, 183; of thyroglobulin, 34, 185; of thyroxine derivatives, 184, 185
Balance, human, 194–200; diarrhea in, 195; diet in, 195; endogenous, 201; essential hypertension, 200; fasting, 200, 201; fecal, 195–200; hyperthyroidism, 197, 200; in blood, *see* Blood iodine; in nontoxic goiter, 197, 209; in toxic goiter, 200, 211; studies of Cole and Curtis, 194, 195 (method), 197–200; studies of von Fellenberg, 195–196; studies of Scheffer, 196–197; and sweat, 195–200; total iodine balance, 196–200, 209, 211; urinary iodine, 196–200, 209, 211, 266
Balance, pharmacological, and iodine excretion, 201–206; and iodide excretion, 201; and diiodotyrosine excretion, 183, 202, 203, 205; and thyroxine excretion, 184, 203; and excretion of organic iodine compounds, 202; excretion by kidney tubules, 202; excretion in feces, 203–205; elimination by liver, 203, 205; tolerance curve, 202, 266
Deficiency, 188; conditioned, 190; and cretinism, 188; and food, 189; and goiter, 137, 147, 181, 182, 188, 251; in mountains, 189; in relation to air, 188, 189; in relation to diet and water, 188,

251; relative, 188, 191, 192; and seaweed, soil, vegetation, 188, 189; and thyroid hyperplasia, 137, 181

Excretion, physiological, 192; after desiccated thyroid, 205; and ovarian cycle, 153; and parathyroid, 143; fecal, 193; in hyperthyroidism, 212; in milk, 70–71, 157, 188, 194; in saliva, sweat, 70–71, 194; total elimination, 194

urinary, 79, 192, 193, 239, 240; after hypophysectomy, 126; after thyroidectomy, 210; from desiccated thyroid, 184, 185, 205; from diiodotyrosine, 193; from thyroxine, 184; in hyperthyroidism, 210, 212

Fetal thyroid, and iodine therapy, 13

Fractions, "D" and "T," 6, 18, 29; endocrine potency of, 29; in blood, 30, 239, 241; in iodoproteins, 29; in thyroglobulin, 30, 44, 61

Metabolism, levels of, 238; after hypophysectomy, 126; fibrolytic level, 268; prophylactic level, 191, 253

therapeutic level, 254; complications of, 256; dosage, 255, 257; escape from, 120, 256; in medical treatment, 256, 260–265; in thyroid storm, 247, 261, 262; mechanism of "Jodbasedow," 251

Methods for estimation of, 271 (*see also* Methods); accuracy of, 275, 283, 284; "D," "T" fractions, 284, 285; electrometric titration, 271, 283; Groák I, 271, 273; Groák II, 271, 275; Hunter-Kendall, 271; in blood, plasma, 286; Riggs-Man, 271, 276

Radioactive, *see* Radioactive iodine

Requirement, 185–188; minimal normal, 186; and nasal secretion, 187; optimal normal, 186; relation to metabolism, 188; relation to retrapping, 186, 210; relation to thyroxine, 186; and sweat, 71, 187, 188, 196; urinary, 186, 187, 188, 192

Thyroid disturbance, iodine balance in, 187, 198, 206; in hyperthyroidism, after excitement, 208, 211; after administration of thyroxine, 208; after iodide feeding, 206; after painting skin, 207; after thyroidectomy, 206; fasting, 206; in cretinism, 207; in feces, 202, 206; in milk, 206; in myxedema, 207; in urine, 206, 207; tolerance curve, 207, 266

iodine metabolism in, 208–212; in bile, 209, 210; in feces, 208, 210; in toxic goiter, 210, 211; in urine, 212; in hypothyroidism, 206, 207

Thyroid iodine, 6, 8, 21, 101, 118, 230; concentration, 16, 101; total in colloid, 9, 10, 230; in gland, 15, 17, 101, 229; in various thyroidal tissues, 11; and thyrotropic activity, 4, 127; thyroxine-like iodine, "T," 6, 15, 18, 22, 25, 58, 74, 75, 285 (*see also* Blood iodine fractions)

Tolerance, in urine, 266

in blood, 81, 259, 266, 268; in euthyroidism, 81, 267; in hyperthyroidism, 267; in hypothyroidism, 207; in nontoxic goiter, 212, 267; with radioactive iodine, 266

Iodine and adrenal cortex, *see* Adrenal cortex

Iodism, 201, 269

Iodo-albumin, in myxedema, 29, 58, 98

Iodo-amino-acids, 50

Iodocasein, 29, 53; "casejodin," 52; "iodocasein" (Liebrecht), 52; "perjodcasein," 52; thyroxine from, 29, 46, 61, 110

Iodoglobin, 29; base binding by, 46, 49; phenolic dissociation in, 50; phenolic groups in, 46; titration curve of, 49

Iodoglutin, 53

Iodogorgonic acid, 7; identification of, 4, 28; in "corals" or sea fans, 7, 28, 31, 32; in thyroglobulin, 28, 50

Iodogorgonin, 32

Iodo-histidine, 7, 28, 50

Iodoproteins, 4; immunity to, 57; iodine numbers of, 54; mechanism of iodination, 55; natural, 31; thyroxine from, 29, 39, 46, 61, 110

Artificial, constitutents of, 52; "D," "T" iodine fractions, 52; diiodotyrosine in, 29, 46; hydrolysis of, 29, 52; iodine in, 46; preparation of, 45; properties of, 46; thyroxine in, 46

Proteolytic cleavage products, "D" and "T" fractions, 57; diiodotyrosine in, 57; endocrine activity of, 58; effect in human myxedema, 59, 60; thyroxine in, 57

Iodospongin, 31, 32
Iodosulfonphenolphthalein test, 66; and liver iodine, 205
Iodopexis, 205
Iodothyronine nucleus, 60
Iodothyrotoxicosis, 122, 251, 256; in cabbage goiter (rabbits), 122
Iodotryptoflavin, 23
Iodozein, acid and base binding capacities, 48; phenol dissociation of, 48; titration curve of, 49
Iopax, 64, 65
Isothyroxine, 111
Isotopes of iodine, radioactive, 213, 215

"Jodbasedow," *see* Iodothyrotoxicosis
"Jodthyrin," 184
"Jodtropon," 52, 100, 104
Junkmann-Schoeller unit, 128, 133, 134, 287–289
Juvenile myxedema, thyroid iodine in, 232

Kaisten, goiter rate of, 188, 189
Kidney, iodide and chloride in, 69; iodine excretion by glomeruli, 202; iodine in, 21, 151
Kitakami, goiter in, 189

"Labeled" iodine, 216; biological behavior of, 219, 227, 233; measurement of, 218, 219, 227, 228; measurement of in thyroid *in vivo*, 231; multiple labeling, 224; preparation of, 215, 218; recovery of, 218
Lactation, and iodine, 146, 191; and thyrotropic hormone, 131, 140
Langley operation, 166, 167
l-diiodotyrosine, peptides of, 12, 30
Leptocardii, thyroid in, 8
Lindbergh apparatus, 103
Lipoidal iodine, *see* Fats
Liquor iodi compositus, composition of, 291; intestinal absorption, 6, 7, 182
Liver, after anesthesia, thyroid feeding, toxic damage, 205; bio-assay of, 205; chloride in, 69; element 85 in, 235; iodide, 69, 204; iodine elimination by, 204; iodine in, 21, 22, 68, 151; organic iodine compounds, 204, 205; thiocyanate in, 68; thyroxine in, 22, 205
Liver disease, in hyperthyroidism, 210
l-thyroxine, peptides of, 12, 30, 60, 115, 117
Lugol's solution, *see* Liquor iodi compositus
Lung, iodide and chloride in, 69; iodine content of, 21, 23, 151; iodine and thiocyanate in, 68
Lupus erythematosus, iodine therapy in, 268
Lymph, chloride, iodine, thiocyanate in, 68, 70
Lymph nodes, element 85 in, 235; iodine in, 229
Lymphadenomatous goiter, 229

Mammillary body, 165
Man, E. B., *see* Riggs-Man method
Marine's cycle, 137; and pregnancy, 147; hypothesis, 224
Maturity, and hypothalamus, 172
Median eminence, 172
Medulla oblongata, iodine content of, 138, 178
Metabolism, after hypothalamic narcosis, 176; in hyperthyroidism, 200; in pregnancy, 155
Metamorphosis, *see* Axolotl; Tadpoles
"Metastable," radioactive atom, 214
Metastases, iodine in, 229, 230
Menopause, and iodine, 152, 191; and ovarian iodine, 149; and thyroid enlargement, 146; and thyrotropic hormone, 152, 153; hyperiodemia of, 93
Menstrual blood, iodine in, 152
Menstrual cycle, and iodine, 146; and iodine metabolism, 151; urinary iodine in, 208
Metabolism, and hypothalamus, 172. *See also* Basal metabolism
Methods, determination of iodine, 270–285; plasma iodine fractions, "I," "P," "T," "D," 286; standard solutions, 291; "T" and "D" iodine, 284; thyrotropic hormone, 286. *See also* Iodine, methods for estimation of
Microdissection of thyroid, 8
Microgram (gamma), definition of, 5
Micrometer, Leitz echelon, 288
Milk, and ovary, 157; iodine in, 70, 188, 201; thyroxine in, 71, 194; vitamin A in whole milk, 190

Millicurie, definition of, 233
Minnesota, simple goiter in, 124
Mitotic activity, in thyroid, 106, 126, 155
Molecular weights of iodine compounds, 290
Monoiodohistidine, 50, 54, 55
Monoiodotyrosine, 7, 50; properties of, 51
Mucous membrane, absorption of iodine, 100, 183
Muscle, element 85 in, 235; iodide and chloride in, 69; iodine in, 6, 15, 21; iodine and thiocyanate in, 68; normal and myxedematous, 18; thyroid hormone in, 18
Myxedema, human, 116, 117; Addison's crisis in, 59, 269; blood iodine fractions, "I," "P," "T," 76, 78, 83, 86, 239, 247; iodinated casein, serum albumin, 18; labeled iodine in thyroid, 232; and menorrhagia, 157; metabolism of radioactive iodine, 227; pituitary in, 126, 144; pituitary iodine in, 145; relief by iodinated proteins, 59, 113; response to thyroxine polypeptide vs. whole thyroid, 117; urinary iodine in, 206, 239
  Thyrotropic hormone, 133, 145; in blood, 133; in urine, 133, 140, 141
  See also Hypothyroidism

Naffziger's operation, in malignant exophthalmos, 260
Neoiopax, 65
Nephritis, excretion of iodine in chronic, 202; excretion of labeled iodine, 228
Nerve tissue, "exquisite" stimulation by thyroid, 161, 165
Nerves, blood iodine after removal of hypothalamus, 179; blood iodine after spinal cord section, 179; peripheral effect on iodine metabolism, 179; to thyroid, myelinated, nonmyelinated, 165
Nervous activity, and Langley operation, 166; interaction with thyroid activity, 167
Nervous system, action on pituitary-thyroid axis, 162; and hypothalamus, 163; effect of bromide and iodide on, 162; effect on iodine metabolism, 160; effect on thyroid, 160, 165; in hyperthyroidism, in hypothyroidism, 161; in iodism, 162
Nervousness, and blood iodine, 151, 194, 242
Neurasthenia, and ovarian cycle, 151
Neurohumoral mechanisms, 162, 171; effect of iodine on, 175
Neutron, production of radioactive iodine, 215
Newborn, ovarian iodine in, 149; thyroidal iodine in, 14
New York, blood iodine in, 79; simple goiter in, 124
New Zealand, iodine in soil, 189
Nitrogen, heavy, 213
Nitroglobin, iodinated, peptic digestion of, 56
Nodular goiter, toxic, 106, 118; case of, 244; nontoxic, iodine in, 210, 211, 229–232; thyroglobulin in, 42, 44
Nucleoprotein in thyroid, 8, 33, 34

Obesity, and hypothalamus, 172
Ohio, blood iodine in, 79
Optic chiasm, 164
Organic iodine, after iodide therapy, 30; "D" and "T" fractions, 31; in blood, 30, 74–76, 79, 86; in hyperthyroidism, 30, 31; in renal clearance, 64; proteins, 27
  Compounds, naturally occurring, 7; storage of, 23; roentgen visualization of, 66
  See also Thyroid gland
Ovarian cycle, and iodine metabolism, 21, 151–153; and menopause, 152; and thyroid gland, 141, 155; in neurasthenia, 151; in thyrotoxicosis, 151, 208
Ovarian extracts, effect on blood iodine, 152; folliculin, 155; lutein, 156
Ovariectomy, effect on anterior pituitary, 155; on gonadotropic hormone, 154; on iodine metabolism, 155; on thyroid iodine, 154; on thyrotropic hormone, 132, 152, 153, 155
Ovary, and adrenal cortex, 3, 156; and endocrine balance, 125; and iodine metabolism, 149; and peripheral tissues, 156; and pituitary-thyroid axis, 156; and thyroid interaction, 154, 157, 158; bio-assay of, nature of, 22, 150; iodine after thyroidectomy, 137; iodine in, 21, 148, 151; trapping of iodine by, 20, 21, 151

Ovulation, and gonadotropic hormone, 175
Oxidation-reduction potentials of iodine compounds, 290

Pallidum, damage of, 176; iodine content, 138, 178; after anterior pituitary injection, 177
Pancreas, and blood iodine, 143; and insulin, 143; iodide and chloride in, 69; iodine in, 21, 22, 143; and thyroid, 143
Parasympathetic nerves, to thyroid, 165, 166; and iodine metabolism, 179
Parathyroid, and iodine balance, 143; iodine in, 21
Paraventricular nucleus, in heat production, 165
Pars tuberalis, and water balance, 172; site of, 163
Pepsin, digestion of iodoprotein, 56, 57; of thyroglobulin, 30, 61
Peptides, iodinated, 58; of thyroxine, 60, 115, 117
Peptones, iodinated, 27, 114
Perfusion of thyroid, 103
Permanganate, reaction with iodide, 271, 273, 275, 290
Phosphorus, in thyroglobulin, 34; radioactive, 213
Phrenic nerve, anastomosis with cervical sympathetic, 166, 167; increased metabolism in, 167
Pilocarpine, effect on blood iodine, 179; effect on thyroid circulation, 168; synergism with thyrotropic hormone, 167
Pituitary, and sympathetic nervous system, 166; effect of thyroxine, 132, 166; release of thyrotropic hormone, 131, 166
Pituitary gland, anatomy of, 163, 164; damage to, 176
  Anterior lobe, 171–175; activity in menopause, 152; after iodine therapy, 122, 133; after thyroidectomy, 19, 137; and gonadotropic hormone, 175; and iodine balance, 125, 126; colloid, 19, 138; and hypothalamus, 19, 177; in menopause, 152; iodine in, 19, 122, 125, 138; portal circulation, 173; protein, 19, 138; veins of, 173; and water balance, 172
  Innervation of, 163; after stalk section, 171, 173; effect of iodine on, 133, 175; from hypothalamus, 173; in cold, 171; and pituitary stalk, 172
  Iodine in, 21, 137, 176; acidophilic granules in, 139; after thyroid feeding, 20, 23, 137, 177; after thyroidectomy, 4, 137; after thyroxine injection, 177
  Middle lobe, after thyroidectomy, 19
  Posterior lobe, 164, 171; after thyroidectomy, 19; and endocrine balance, 125; and supra-optic nucleus, 164; and water balance, 173; atrophy after nerve section, 172; iodine in, 19; lesions of, 176
Pituitary-ovarian axis, 139, 146; and thyroid, 156
Pituitary-thyroid axis, 131, 153, 156, 171; and "castration phenomenon," 132, 145; effect of thyroid hormone, 131, 132, 137; effect of thyroxine, 132; and menopause, 132, 152; and myxedema, 133, 141; peripheral relationship, 144
Pituitary-thyroid interaction, 139; effect of eosinophilic elements, 171; and hypothalamus, 139, 167, 173, 177; and stalk section, 167, 171; and thyroid hyperplasia, 137, 167
Pituitrin, and diabetes insipidus, 172; and iodine balance, 172
Placental blood iodine, 153; circulation of iodide, 13; circulation of thyroid hormone, 14
Plasma fractions, "I," "P," "T," "D," 75, 239–241, 286; iodine, "I," 255; iodine vs. whole blood iodine, clinical data, 76
Plastein, 61, *see* Thyroid preparations
Pluriglandular syndrome, case of, 246
Portal vein, iodine in, 205
Potassium iodate, standard solution, 291. *See also* Iodate
Potassium iodide, iodine content of, 290; molecular weight of, 290. *See also* Iodide
Precipitin reaction, in blood to thyroglobulin, 70, 75, 99, 185; in thyroid lymph, 70
Pregnancy, and goiter, 146, 154, 191; and thyrotropic hormone, 140; effect on thyroid, 154, 191, 222; iodine excretion, 153, 154; iodine requirement, 186
Pre-optic region, 164
Proteins, iodinated, 7, 18, 27, 45, 113;

calorigenic effect, 113, 114; cardio-accelerating effect, 114; "D" and "T" fractions, 58, 113; methods of iodinating, 28; split products of, 113
Proteolytic enzymes, 27; effect on thyroglobulin, 27; erepsin, pepsin, trypsin, 30
Proto-albumose, iodination of, 53; properties, 54
Pseudopregnancy, and gonadotropic hormones, 175
"Psychosomatic medicine," 173
Puberty, and goiter, 146; and iodine, 146, 186, 191
Putamen, damage to, 176

Quotient, *see* Blood iodine, fasting

Radiation therapy, 90, 210, 260, 262; with radioactive iodine, 233
Radioactive iodine, absorption of, 100; diffusion through body tissues, 219, 220; excretion of, 219, 228; human metabolism of, 226–228; in blood, 214, 219; in body fluids, 219; in body tissues, 219; in carcinoma, 229, 230; in cerebrospinal fluid, 219; in feces, 214, 217, 226, 227; in hyperplastic thyroid, 220, 221 (Type 1, 221–225; Type 2, 221–225); in metastases, 229, 230; in thyroid, 217, 219, 220, 229–233; isotopes, 215; storage in thyroid, 100
Radio-autograph, 230
Reaction time, and thyroid, 161
Red cells, *see* Erythrocytes
Refractoriness to iodine, 120, 121
Renal clearance, of organic iodine compounds, 64; tubular excretory mass, 66
Riggs-Man method of iodine determination, 271, 276
Roentgen therapy, 260; and blood iodine, 90, 262; and iodine balance, 210
R unit, definition of, 233

Saliva, iodine in, 71, 194, 201, 268
Schoeller unit, *see* Junkmann-Schoeller unit
Sea fans, 7, 28; hydrolysis of, 28; iodine in, 27; iodogorgonic acid in, 28
Seaweed, diet, effect on thyroid, 102, 189; iodine in, 32
Serum, chloride in, 69; iodide in, 69
Serum albumin, iodinated, 55; in human myxedema, 18, 59, 98
Serum globulin, iodinated, 55
Sex, and endemic goiter, 146
Sex function, and hypothalamus, 172; and iodine, 146
"Shotgun reaction" of pituitary, 154, 156
Simple goiter, 123, 124; basal metabolism in, 86, 124; bio-assay of, 123; blood iodine fractions, 94, 239 ("I," "P," "T," 239, 241); case of, 241; follicles in, 123, 251; hypothyroidism in, 124; iodine prophylaxis of, 192, 253; iodine stores in, 12, 123, 210, 211; plasma iodine in, 239; relative iodine lack, 123, 190, 191; thyroid medication in, 252; thyroid parenchyma in, 123; tyrosine in, 124; urinary iodine in, 210, 239
Skeletal muscles, iodine in, 6, 15, 21; thyroid hormone in, 18
Skeleton, iodine in, 21
Skin, absorption of iodine, 100, 207; iodide and chloride in, 69; iodine in, 21, 23, 68; thiocyanate in, 68
Skiodan, 64, 65
Sleep, and hypothalamus, 172
Small intestine, iodine in, 21
Sodium thiosulfate, standard solution, 291
Soil, iodine content of, 188
Spleen, iodide and chloride in, 69; iodine in, 21, 68; thiocyanate in, 68
Sponge, burnt, of ancient Greeks, 27; diiodotyrosine in, 7; iodine in, 27
Starch, reaction with iodine, 290
Stomach, absorption of iodine compounds by, 182; iodide and chloride in, 69; iodine in, 21; iodine excretion by, 23, 81, 203
Storm, thyroid, 85, 91, 261; case of, 247
Struma lymphomatosa, iodine in, 229–230
Submaxillary gland, iodine in, 21
Superior cervical ganglion, 165
Superior laryngeal nerve, 165
Superior thyroid arteries, 165; ligation of, 91
Supra-optic nucleus, 164; after section of pituitary stalk, 172; and water balance, 164, 173
Surgery, and iodine excretion, 194, 210
Sweat, iodine in, 71, 194, 195, 208
Switzerland, iodine in soil, 189

Sympathetic nerves, and gonadotropic hormone, 174, 175; and hypothalamus, 163, 164, 171; increased thyrotropic hormone from, 162; stimulation of pituitary and thyroid by, 162; to thyroid, 165

Sympathin, liberation on sympathetic stimulation, 171

Syphilis, iodine therapy in, 268

Tadpoles, metamorphosis of, 8, 112, 151; method, detection of thyroid activity, 19

Tagged atoms, radioactive iodine, 213, 216, 217; biological behavior, 217, 232

Tellurium, source of radioactive iodine, 215, 218

Testicle, iodine in, 21, 22, 151

Tetrabromthyronine, 111, 112

Tetraiodothyronine, *see* Thyroxine

Thalamus, after injection of anterior pituitary extract, 177; damage to, 176; iodine content of, 138, 178

Theelin, 156; and cancer, 157; and pituitary-ovarian axis, 156

Thiocyanate, and iodine metabolism, 191; in lymph, 68, 70

Thiosulfate, titration of iodine, 272, 274, 275, 281, 290. *See also* Iodimetry

Thirteen-day iodine, 214

Thoracoplasty, and iodine excretion, 194, 210

Threshold for iodine storage by thyroid, 221, 222

Thymus, 143; iodine in, 21; in hyperthyroidism, 144; and thyroid, 144; and thyroxine, 144

"Thyranon," 116

"Thyrine," 234

Thyroglobulin, 4; aggregation of molecule, 37; calorigenic potency of, 185; carbohydrate in, 38; composition of, 34; constituent amino-acids of, 37, 39, 40, 44; diiodotyrosine in, 25, 38, 39; glucosamine in, 38; hydrolysis of, 24; immunity to, 75, 99, 129; in hyperthyroid glands, 43; in normal thyroid glands, 40, 42; in pathological thyroid glands, 42; iodination of, 27; iodine in, 11, 34, 39; isoelectric zone, 35, 37; molecular architecture, 35, 37, 40, 41; preparation of, 32; properties of, 34; pure, 11; salting out of, 35; sedimentation constant, 36; specific volume, 36; thyroxine in, 24, 38, 39

Peptide and peptone of, 61; assimilation, 185

Plastein of, 61; effect in myxedema, 63; preparation of, 62; properties of, 63

Thyroid, human, iodine in, 6, 8, 10, 12, 15, 16, 17, 101. *See also* Thyroid gland

Thyroid cells and parenchyma, ratio of iodine in, 9; "concentration coefficient," 220, 221; effect of ovulation, of sex, 157; follicles, 8; secretion of colloid, 9

Thyroid gland, activity, 100; after theelin injection, 154, 156, 157; blood flow of, 167, 168; cancer of, iodine in, 229, 230; cancer protein, 13; carcinoma, radiation therapy with radioactive iodine, 233; effect of cold, 106; effect of iodide administration, 105, 122, 133; "endocretion," 106, 120; and endocrine balance, 125; evolution of, 8; hyperplastic tissue, 8, 220, 221 (Types 1 and 2, 221–225); hypertrophy, 147 (in estrus, 155; in pregnancy, 155); effect of hypophysectomy, 126; in beriberi, rickets and scurvy, 190, 191; lymph of, 70; in menopause, 152; iodide in, 7, 17, 24, 120 (after iodide therapy, 24, 25, 151, 217, 219–222, 224–227, 229–232); and age, 15; iodine stores, 104, 118, 210; in hyperthyroidism, 118; microdissection of, 8; mitotic activity, 106 (in ovarian cycle, 155); nerves of, 165–166; nucleoprotein in, 8, 33; and other endocrines, 141; ovarian activity, 141, 154; parenchyma of, 9; and pituitary-ovarian axis, 156; after injection of progesterone, 154, 156; "reserve" of iodine, 8; secretion of active, 18; secretion of inactive, 8; "secreting" tissue, 8; storm, 91, 247; stroma, 9; "T" fraction, 17, 230, 231; effect of thyrotropic hormone and theelin, 154; thyroxine in, 25, 101, 120; effect of thyroxine administration, 167; trapping of radioactive iodine, measurement *in vivo*, 220, 231; weight of, 15, 16

Colloid, deposition of, 120; "inactive," 8; iodine in, 25, 230; release of, 16; stores in, 16, 107, 230; types of, 8

"D" fraction, 17, 25, 120, 230, 231; "D" and "T" fractions in, 17, 118

Follicular cells, 9, 106, 121, 133, 141, 289; effect of cervical sympathetic nerves, 168, 169; chemical control of, 169, 170; effect of cold, 168, 170; and follicles, 121; height of, 141, 168, 287, 289; in human pathological states, 289; iodine in, 25, 230; mitochondria of, 167; effect of nerve stimulation, 167; polarity of, 106, 121; effect of thyrotropic hormone, 11, 127, 168, 287; thyroxine in, 25

Hormones, natural, 114; multiple hormone theory, 115; unknown iodine compounds, 113. *See also* Thyrotropic hormone

Hyperplasia, 123, 137, 147, 186, 230; after ovariectomy, 154; and vitamin A, 191, 192; dual cause, 224; and estrus, 155; in pregnancy, 155; involution, 147; radiation therapy with radioactive iodine, 233

Iodine content, 6, 12, 21, 101, 119, 120; average in, geographical distribution, 16; "D" and "T" fractions, 17, 25, 61, 120, 230, 231; diiodotyrosine in, 4, 24, 25, 50, 101; geographical variation, 101; heat coagulable, 24; hibernation, effect of, 103; in Argentine sheep, 101; in fish diet, 102; in human newborn, 14; in human thyroids, 17, 101; in New York, 16; in newborn hogs, sheep, 14; in normal adult, 101; in normal thyroids, 15; in ovarian cycle, 155; lipoid iodine, 24, 101; organic iodine in, 130; radioactive iodine in, 24, 214, 229; seasonal variation, 101; effect of sex activity, 103, 158; thyroxine in, 24, 25, 101; total, 120; effect of ultraviolet light, 103; effect of vitamin D, 103

Thyroid hormone, bodily store of, 108; circulating, 18; daily requirement of, 107; in pregnancy, 155; placental circulation of, 14. *See also* Thyroid gland, hormones

"Thyroid index," 147, 220

Thyroid medication, manufacture of, 109, 110; activity in blood serum, kidney, liver, 19; and gonadotropic hormone, 158; cardio-accelerating effect, 114; effect of added iodide, 122, 252; effect on tissue iodine, 23, 177; ovarian effect of pituitary transplants, 158

Thyroid nerves, 165–167; blood vessels, 165; lymphatics, 9, 70

Thyroid preparations, calorigenic potency of, 115, 116, 185; cardio-accelerating effect of, 116; effect of "D," "T" fractions, 115; desiccated thyroid and thyroglobulin vs. thyroxine, 116, 185; and liver iodine, 205; potency of plastein, 63, 117; potency of thyroxine vs. total iodine, 109

Protein, artificial, assimilation of, 185; cancer protein, 13; deiodinated, 9; preparation of iodinated, 32; synthesis of, 117; uniodinated, 8

Thyroidal colloid stores, 9

Thyroidectomy, and iodine in body tissues, 137; and vitamin A, 190; effect on blood iodine, 92, 149; inhibition of breast, 155; effect on growth, 144; in pregnancy, 13; iodine excretion after, 194, 210; inhibition of lactation, 155; effect on ovarian iodine, 151; effect of thyroid feeding, 137; thyrotropic hormone, effect after, 140

"Thyroidin," 20, 184

"Thyrojodin," 184

Thyronine, 111; derivatives of, 112

Thyrotoxicosis, *see* Hyperthyroidism; Graves' disease

Thyrotropic hormone, and acetylcholine, 168; effect of adrenals, 126, 128; after iodide, 122, 128, 133–136; after thyroidectomy, 140; antagonism of iodide, 128, 134, 136; antibodies to, 129; antihormones to, 129; assay of, 128, 133, 286; and basal metabolic rate, 127, 133, 139; and blood hormone, 131, 132, 139; and blood iodine, 129, 130, 132, 133; effect on blood iodine fractions, "P," "T," "D," 130; and cabbage goiter, 140; and circulating iodine, 140; control of, 131, 139, 175; and crystalline sex hormones, 128; effect on denervated thyroid gland, 175; and dibromtyrosine, 137; and diencephalon, 139, 173; and diiodotyrosine, 135, 137; discovery of, 125; and endocrine balance, 125; and ephedrine, 168; and exophthalmic goiter, 160, 260; and exophthalmos, 127, 260; effect on follicular cells, 126, 127, 133, 287; and goitrogenic diet, 140; and gonadotropic hormone, 128; and growth, 144; and growth hormone, 128; and heart rate, 127; and histological changes, 126, 128, 133, 289; hyperplasia of thyroid gland, 131, 224; and clinical hyperthyroidism, 86, 128, 139; immunity to, 93, 129; in birds, fowl, fish, guinea pigs, 127; in blood, 133, 139; in cold, 131, 140; in cyanide poisoning, 131, 140; in fear, 131; in hunger, 131; in lactation, 131, 140; in menopause, 152, 153; in pregnancy, 131, 140; in urine, 133, 139, 141; effect of *in vitro*, 129; and infection, 140; and iodide dosage, 137; and iodine balance, 139; iodine lack, 140; effect on isolated thyroid, 129; Junkmann-Schoeller unit of, 128, 289; and Marine's hypothesis, 131; effect on metabolism, 125; and myxedema, 131, 133, 140; neurohumoral relationships, 131; of non-pituitary origin, 126, 127; effect of ovary, 126, 128, 154; and peripheral effect of iodide, 134; physiological effects of, 126; and pilocarpine, 168; and pituitary-thyroid axis, 131, 132, 137; and progesterone, 154, 156; and radioactive iodine, 225; refractoriness to, 129; and sexual excitement, 131; and sympathetic nerves, 129, 131, 171; synergism of iodide, 134, 136; and thyroidal "T" fraction, 129, 130; effect on thyroid chloride, 127; effect on thyroid colloid, 11, 127, 130; effect on thyroid gland, 126, 127, 130, 131, 139; effect on thyroid iodine, 128, 130; and thyroid medication, 140; effect on thyroid transplants, 175; effect on thyroid vessels, 127; and distribution of water in thyroid, 11, 127; and urinary iodine, 130; and vitamins, 103, 190; and young chicks, 287

Thyroxamine, 112

Thyroxine, 4; acetyl derivatives of, 112; amino group of, 112; assimilation of, 184; calorigenic activity, 110, 112, 185; circulating in blood, 18; crystalline, absorption of, 184; derivatives of, 23, 112, 115, 185; in gastrointestinal tract, 184, 185; and diiodothyronine, 111; d-thyroxine, potency of, 111, 185; esters of, 112; evolution of, 7, 8; effect in human myxedema, 18, 117; in feces, 184, 203; in fetal thyroid, 14; in hypothyroidism, 118, 208; in milk, 70, 194; in synthetic thyroxine protein, 118; in thyroid gland, 120; in thyroid of newborn, 15; in urine, 208; iodine content of, 51, 291; iso-thyroxine, 111; l-thyroxine, potency of, 111, 185; metabolism of, 203; and metamorphosis, 112; molecular weight of, 291; peptide of, 18, 112, 115; potency of peptide, 116, 118, 184, 185; peptones of, 185; perfusion through thyroid gland, 104; effect on pituitary-thyrotropic content, 167; polypeptide of, 117, 118, 185; precursor of, 26; properties of, 50, 51; racemic, absorption of, 116, 184; potency of racemic, 111; sodium salts of, 184; solubility in biological fluids, 184; storage of, 23; effect on thyroid gland, 167; urinary iodide from, 184, 203

Thyroxyl-diiodotyrosine, 115

Tissue extracts and metamorphosis, 19, 205

Tissues, bio-assay of, 18, 205; iodine in (e.g., "D," "T," and total), 18

Tolerance, iodine, *see* Iodine, tolerance

Transudates, chloride and iodine in, 69

Trapping of iodine, after thyroid feeding, 23; by ovary, 21, 151; by thyroid, 24, 210, 212, 220, 221, 229–232; in liver, 23, 204; threshold for thyroid, 221–225

Trypsin, digestion of iodoprotein, 57; of thyroglobulin, 30, 61

Tuber cinereum, 138, 173; after anterior pituitary extract, 177; after diiodotyrosine, 177; after hypophysectomy, 20, 177; after iodide, 177; after thyroid feeding, 20, 23, 177; after thyroxine, 23, 177; damage of, 176; and endocrine balance, 125; iodine in, 20, 138, 177, 178; site of, 163, 164; thyroid hormone, selective absorption by, 20, 177

Tubero-hypophysial nerve tract, 173; site of, 163, 164

Twenty-five-minute iodine, 215, 216, 220
Tyrosine, calorigenic effect, 112; derivatives of, 47, 112; iodination of, 28, 47; phenolic dissociation of, 47

Ultracentrifuge, 35, 36
Umbilical blood iodine, 153
U. S. P. desiccated thyroid, 16, 116, 291
United States, iodine in soil, 189
Uranium, source of 8-day iodine, 216
Urinary iodine excretion, 79, 126, 210, 239, 240. *See also* Iodine, excretion
Uroselectan, 64
Uterus and fallopian tubes, iodine in, 21

Vasomotor changes in thyroid, 167, 168
Vegetation and urinary iodine, 192
Vienna, goiter in, 251
Viscera, iodine in, 22
Vitamins, and goiter, 191; and iodine metabolism, 103, 190, 191; and thyroid gland, 103, 190–192; and thyrotropic hormone, 103, 190

Water, and urinary iodine, 192; drinking water and goiter, 188; iodine in body water, 11, 67, 127
Water and electrolytes, distribution of between cells and colloid, 10, 11, 127; in goiter, 11
Water balance, and diencephalon, 164, 172, 173; and pituitary, 173
Wet-ashing, 270, 276

Xenon, from radioactive iodine, 216
X-ray, *see* Roentgen therapy

TECHNOLOGICAL RESEARCH INSTITUTE
LIBRARY

CFTRI LIBRARY, MYSORE – 570 013

Acc. No.

Call No.

Please return this publication on or before the last due date stamped below to avoid incurring overdue charges.

To be issued from:

DUE DATE	RETURNED ON	DUE DATE	RETURNED ON

✓